Effect of Chemical Seed Treatments, Mycorrhizae, *Rhizobium* and Pelleting of Shrub Seeds Used in Disturbed Land Reclamation

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between

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ABSTRACT

Effect of Chemical Seed Treatments, Mycorrhizae, Rhizobium and Pelleting of Shrub Seeds Used in Disturbed Land Reclamation.

It is well supported that plant growth and establishment in cropping systems is dependent to a large degree on seed quality and germinability early in the life of the plant as well as symbiotic association between the plant and soil microorganisms a little later in plant development. Consideration of these two parameters as they relate to reestablishment of native vegetation has received much speculation but at present little research. The research reported here is an initiation of work directed towards increasing knowledge in these two areas.

Chap. I, Sec. 1

A review of literature pertaining to vesicular-arbuscular (VA mycorrhizae and severe land disturbance was conducted. Early work demonstrated that VA mycorrhizal infection often resulted in host growth enhancement. This effect is thought to be primarily due to the phosphorus uptake function of the VA mycorrhizae; although plants do differ considerably in the extent to which they depend on VA mycorrhizae mediated P uptake. Numerous researchers reported presence of VA mychorrhizae on plants colonizing mine wastes. However, perhaps more important, numerous researchers have shown that severe land disturbance also disturbs VA mycorrhizal fungi: (1) infective potential of the soil and

propagule numbers are modified downward, (2) long term soil storage further reduces VA mycorrhizal infective ability of soil, and (3) mycorrhizal fungi recolonize soils only very slowly. Several research groups have demonstrated improved growth of desirable reclamation species through plant inoculation with VA mycorrhizal fungi. This observation coupled with observation that land disturbance promotes establishement of non-mycorrhizal plants and selects against mycorrhizae dependent plant. Applied use of VA mycorrhizal fungi in mined land environments appears to be related to (1) preservation of the soil mycorrhizal flora, (2) inoculation of soil, and (3) inoculation of container stock.

Chap. I, Sec. 2

Root proliferation and development of vesicular-arbuscular mycorrhizae in western wheatgrass (Agropyron smithii Rydb.) was investigated at a revegetated surface mine in southeastern Wyoming. Total rooting density (root length per volume of soil), infected root density, percentage infection, and spore frequency increased as functions of site age (time elapsed since initial revegetation). In the most recently revegetated sites. The abundance of roots and mycorrhizae of seedlings was positively correlated with extractable soil P. Topsoil replaced during reclamation was a primary incoulum source. Seedlings growing on spoil material without topsoil were generally non-mycorrhizal, but mycorrhizal rooting density was not significantly correlated with residual soil organic matter content (an indicator of the proportion of topsoil in the growth media), possibly due to inoculum depletion caused by prolonged topsoil storage. Mycorrhizal development in sites up to three years old

was minimal in spite of rapid increases in overall rooting density. Rooting density and infection levels in older sites (5-7 years) were comparable to those of an adjacent undisturbed prairie site, but were highly variable. Mature non-mycorrhizal specimens were observed indicating that A. smithii is not necessarily dependent on mycorrhizal infection for survival on severely disturbed lands. Other more dependent species could have been adversely affected by the scarcity of inoculum during early revegetation.

Chap. II, Sec 1

Seed dormancy was characterized in Gardner saltbush (Atriplex gardneri (Moq.) D. Dietr.) big sagebrush (Artemisia tridentata Nutt.), green and rubber rabbitbrush (Chrysothamnus spp.) and Siberian peashrub (Caragana aborescens Lam.) using local collections or commercial seed sources. Gardner saltbush was emphasized. A high level of seed dormancy was found in this species. Treatments used to alleviate seed dormancy in an effort to further characterize it included stratification, washing, and scarification of seed held in dry storage for 2, 5 and 15 months post harvest. Increased time of dry storage afterripening partially ameliorated dormancy. Four week stratification generally enhanced germination. Washing, alone, was somewhat less effective, but still beneficial. Scarification alone enhanced germination after 2 and 5 months dry storage, but primarily rendered the seed more responsive to stratification and washing treatments. After 15 months of dry storage afterripening, seed dormancy of Gardner saltbush seed was completely broken by a combination of 4 week stratification, 24 hour washing and scarification. These results suggest that complete

dormancy removal may require physiological embryonic development from stratification and dry storage afterripening, and removal of seedcoat and/or germination inhibitors by washing and scarification. Seed dormancy was absent in the sagebrush seed evaluated, and totally alleviated by a short period of stratification in Siberian peashrub. Rabbitbrush seed evaluated was of poor quality, but apparently was characterized by a moderate level of seed dormancy.

Chap. II, Sec. 2

To assess the practical value of the Gardner saltbush seed treatments discussed in Section 1, field plantings were made on two mine reclamation sites and on an irrigated site at Laramie, WY. Preliminary studies showed that germination enhancement obtained from stratification and wash treatments was retained by seed allowed to air dry up to 2 and 10 days respectively. This implied that in field application the treatments would not have to be applied immediately prior to planting to retain their benefit.

Field emergence results did not correspond exactly with laboratory germination results, although emergence on the mine sites was so low as to make conclusions tenuous. Under irrigation stratification of seed prior to planting provided enhanced emergence, which was further stimulated when combined with scarification. These studies demonstrated the necessity for extending laboratory research to the field.

Chap. II. Sec. 3.

Gardner saltbush seeds collected from different geographic sites and in different years were compared for germination response with and

without the treatments discussed in Section 1. Significant differences in germination of stratified and washed seed from different sites was observed. The difference was reduced by scarification. Seed collected at the same site in different years showed variation in percent germination, but generally responded to scarification, washing, and stratification treatments similarly. Some indications of variability in depth of dormancy between years was noted.

PUBLICATION LIST

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FUTURE DIRECTIONS

Research on endomycorrhizal enhancement of shrub survival and establishment is continuing through a project funded by the University of Wyoming Industrial Fund ($8,000 for 7-82 through 6-83) and through similar funded projects from the Department of Transportation and the Department of Energy. Currently a large number of encomycorrhizal cultures have been established and are being maintained. During the summer of 1983 plantings of inoculated shrubs will be made at sites in the Snowy Range of southeastern Wyoming and the Shirley Basin of southeastern-central Wyoming. Growth and survival of these plants will be monitored.

Research with Gardner saltbush seed is continuing at a minimla funding level on Wyoming AES Match funds available for the project. Germination responses to the seed treatments described in this report using 1980 seed collections are being obtained on seed collected in 1981 and 1982. The basis for the enhanced germination obtained by washing treatments is being studied, and a red pigment formed in radicles of germinating scarified seed but absent in non-scarified seed will be characterized. The long-term survival of seedlings obtained in the field seeding study will also be monitored. Additionally, the germination ecology of Gardner saltbush will be studied by comparison of soil characteristics of seed collection sites with germination response of the seed from those sites. The long-term objective of this seed research is to increase our knowledge on the effect seed sources (ecotypes) of a species have on successful revegetation of diverse sites.

CHAPTER I

ENDOMYCORRHIZAL COLONIZATION OF A SURFACE MINE

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SECTION I

VESICULAR-ARBUSCULAR MYCORRHIZAE AND SEVERE LAND DISTURBANCE

Contents

1.A. Introduction

Among the many types of microorganisms which inhabit the soil and the roots of plants, the fungi are especially notable. Soil fungi perform vital processes like litter decomposition, but they also incite devastating plant diseases. Fungal pathogenesis is only one of several types of intimate interactions between soil fungi and higher plants. A prolonged, nutritionally interdependent, intimate relationship between organisms is called a symbiosis. Symbioses range from antagonistic interactions to those which are beneficial to both symbionts (mutualism). Symbiotic soil fungi inhabit the rhizosphere surrounding plant roots and the roots themselves (endophytic symbiosis). They derive nutrition either from organic substances in the soil (saprotrophism), from dead tissues (necrotrophism), or from living tissues (biotrophism).

A mycorrhiza ("fungus-root") is a type of endophytic, biotrophic mutualistic symbiosis which is prevalent in many cultivated and natural ecosystems. Because they are very common and frequently beneficial to plant growth they have recently received a great deal of attention. Part of this has been stimulated by the acceleration of severe land disturbance; mycorrhizae are present in many currently and potentially disturbed areas and they seem to be particularly sensitive to such impacts. Perhaps most important, they appear to play a fundamental role in the recovery of disturbed lands.

This article includes a brief introduction to the most common type of mycorrhizae. It is a review of the published research regarding the effect of severe disturbance on this type of symbiosis and its influence on ecosystem recovery.

1.B. Vesicular-Arbuscular Mycorrhizae

There are three major groups of mycorrhizae, based on infection morphology and endophyte taxonomy (Harley, 1969; Cook, 1977; Smith, 1980).

The ectomycorrhizae are associations of higher fungi (Basidiomycetes and Ascomycetes) with the roots of woody perennials, especially those in the coniferales (Marks and Kozlowski, 1973). The fungi form intercellular ramifications of mycelium in the host cortex called Hartig nets and dense hyphal encapsulations of the fine roots known as sheaths, or fungal mantles. Ectomycorrhizae are most important in forested ecosystems.

Endomycorrhizae are characterized by fungal penetration of the host cells. There are several types (Smith, 1980) in two major groupings (Harley, 1969; Cooke, 1977).

Endomycorrhizae with septate endophytes occur in the Orchidaceae and the Ericales. The endophytes are higher fungi (Sanders, Mosse and Tinker, 1975; Cooke, 1977).

Endomycorrhizae with generally aseptate endophytes are the "phycomycetous" or vesicular-arbuscular (VA) mycorrhizae (Mosse, 1973; Gerdemann, 1975; Cooke, 1977; Mosse, 1981). The fungi belong to the family Endogonaceae of the order Mucorales and the class Zygomycetes. Four genera engage in known VA mycorrhizal interactions: Acaulospora Gerd. and Trappe, Gigaspora Gerd. and Trappe, Glomus Tul. and Tul., and Sclerocystis Berk. and Broome (Gerdemann and Trappe, 1974). Little is known of the phylogeny or life cycles of these organisms, primarily

because pure culture methods haven't yet been developed. These would greatly enhance research efforts (Mosse, 1981).

Gross root morphology is not usually altered, but the internal morphology of VA mycorrhizae is higly characteristic. The fungi invade host roots and proliferate inter- and intra-cellularly in the root cortex, where they form vesicles (terminal hyphal swellings which function as storage organs and propagules) and arbuscules (intracellular dichotomous haustoria). External hyphae grow from the root and bear additional vesicles and resistant spores, frequently in hypogeous sporocarps (Gerdemann, 1975; Mosse, 1981). Details of ultrastructure are in Holley and Peterson (1979) and Abbott and Robson (1979).

Infection can cause a beneficial physiological effect on host plants, mainly by enhancing uptake of soil phosphorus. The presence of the external mycelium causes a greater volume of soil to be exploited, which overcomes a "phosphate depletion zone" about the root (Bowen, Bevege and Mosse, 1975; Tinker, 1975 a,b; Rhodes and Gerdemann, 1978). Host growth is frequently enhanced, particularly in P deficient soils (Gerdemann, 1968; Mosse, 1973). The effect is summarized by Mosse (1981):

1. Plants differ in the extent to which they depend on mycorrhizal uptake.
2. The extent to which a plant benefits . . . depends on the degree of P deficiency the non-mycorrhizal plant is experiencing in that environment and on the reserves of available soil P.

3. The extent of mycorrhizal development is affected by the plant nutrient level. This latter effect is probably due to changes in root membrane permeability caused by enhanced tissue P concentrations (Graham, Leonard and Menge, 1981). Infection causes a carbohydrate drain on the host (Ho and Trappe, 1973; Kucey and Paul, 1981), but in low P environments the exchange of carbohydrate for inorganic minerals can be highly favorable to the host (Bevege, Bowen and Skinner, 1975). Other immobile elements may be taken up as well. Other changes (e.g. water relations, phytohormone levels, etc.) attributed to infection may be secondary effects of improved P nutrition (Rhodes and Gerdemann; 1978; Moss, 1981).

Infection occurs in the bryophytes, pteridophytes, gymmosperms, and in most angiosperms (Gerdemann, 1975). It occurs in all terrestrial biomes. Many communities are essentially devoid of non-mycorrhizal plants, Including many grasslands and steppes of arid western North America (Gerdemann and Trappe, 1974; Williams and Aldon, 1976; Christensen and Williams, 1977; Davidson and Christensen, 1977; Taber and Pettit, 1977; Molina, Trappe and Strickler, 1978; Reeves et al., 1979; Miller, 1979; Williams, 1979; Stahl et al. 1979; Stahl and Christensen, 1982). The major food grains and most other crop species are hosts (Mosse, 1981).

There organisms appear to have a significant role in ecosystem function (Baylis, 1967), but very little synecological research has been conducted, particularly in semi-arid and arid lands (Trappe, 1981). Fogel (1980) emphasized the need for information regarding their role in

nutrient cycling. They are likely to be a primary mechanism of P uptake from soil, and they may take up nutrients directly from litter (Went and Stark, 1968; Stark, 1977). Evidence for decompositional activity is sparse, though they have been observed exploiting rich organic microsites (St. John and Coleman, 1981). Translocation through hyphal connections between hosts has been observed (Heap and Newman, 1980 a, b; Whittingham and Read, 1982). Infection can influence host susceptibility to root disease (Schonbeck, 1979).

This evidence indicates that vesicular-arbuscular mycorrhizal fungi influence mineral cycling, water relationships and perhaps both primary productivity and competition in terrestial ecosystems, though the extent to which they do so is not yet clear. They may also affect seedling establishment, community composition and the rate of succession during the recovery of disturbed land. They are clearly affected by certain land disturbances, and the relationship between endophyte communities and severe disturbance has received considerable attention.

I.C. The Effect of Severe Land Disturbance on VA Mycorhizae

For this discussion, "drastic land disturbance is such that ". . . the native vegetation and animal communities have been removed and most of the topsoil is lost, buried, or altered." (Box, 1978). This includes mined lands, construction sites and seriously eroded lands. Population pressures, increased living standards and massive agricultural exports have dramatically accelerated the rate of land disturbance in North America in spite of advances in soil conservation and reclamation.

Serious perturbation of an ecosystem will affect its symbiotic interactions. Man-caused disruptions of soils and plant communities can affect populations of VA mycorrhizal fungi. Christensen and Williams (1977) and Stahl et al. (1979) found that levels of infection and propagule numbers were much higher in undisturbed prairie than in surface mined sites, especially in raw spoils which had no topsoil applied. Allen and Allen (1980) found low infection levels and spore counts in topsoiled surface-mined sites up to three years old. Even minor disturbance (i.e. disking) reduced infection levels compared to those of adjacent undisturbed prairie. Reeves et al. (1979) found extensive infection in undisturbed sites in western Colorado. Infection in a disturbed site (an abandoned roadway) was negligible. Effective inoculum density was much lower in the disturbed area (Moorman and Reeves, 1979) In a later study (Reeves, 1979), more severe types of disturbance (e.g. burial of topsoil vs. removal of vegetation) caused progressively greater losses of "mycorrhizal infection potential." Miller (1979) found no mycorrhizae in stripmined sites in a very harsh

area of Wyoming, even though adjacent undisturbed desert communities were extensively infected.

Removal or deep burial of the soil can result in surface media with essentially no mycorrhizal fungi. Danielson, Zak and Parkinson (1979) and Zak and Parkinson (1981) found little or no infection in young Agropyron spp. growing in untreated coal mine spoil and tar-sand waste. When topsoil is not salvaged prior to overburden excavation and becomes mixed with the spoil material, a dilution of the soil inoculum occurs. Ponder (1979) found that plants growing in spoils so treated became mycorrhizal, but the development of infection in such situations is variable (Walker and Bartlett, 1981). Infective potential can be reduced markedly by erosion as well (Powell, 1980).

Topsoil salvage and post-mining replacement provides inoculum for seedlings and simulates the pre-mining edaphic conditions of the site. Long-term storage of topsoil can deplete its inoculum potential. Rives et al. (1980) saw diminished infective potential in soils stored for three years. Christensen and Allen (1979) recovered relatively few spores from stockpiles over five years old. Williams, Loree and Singleton (1981) noted that topsoil infective potential was negatively correlated with stockpile age. Moorman, Schmidt amd Miller (1979) also detected reduced infective potentials and low spore numbers in stored soils. Apparently, VA mycorrhizal fungal spores can endure storage to some extent. However, a major part of the inoculum present in undisturbed soils is in the form of hyphae from existing mycorrhizae (Read, Koucheki and Hodgson, 1976) and this component is rapidly lost (Rives et al., 1980).

Endophytic fungi may not readily adapt to chemical and physical changes in the soil caused by disturbance, especially when it involves dilution or contamination of the soil. Miller (1979) found that infection did not develope in a disturbed Wyoming desert site in spite of the presence of viable inoculum. Edaphic conditions which caused salt accumulation inhibited both infection and sporulation in these soils (Miller, 1980, 1981). Schwab and Reeves (1979) found that infection potentials of Colorado soils were greatly reduced by the addition of retorted oil shale wastes.

Vesicular-arbuscular mycorrhizal fungi seem to colonize new areas slowly (Gerdemann and Trappe, 1974; Hosse, Hayer and Clarke, 1981), due in part to the large size and subterranean formation of their propagules. This, in combination with the substantial reductions in endophyte populations caused by disturbance, can result in areas which have minimal populations for long periods. Since: ". . . the sensitivity of such associations can have important implications to ecosystem resistance to gross stresses and to the recovery therefrom . . ." (Harrison, 1971), the role of these organisms in system recovery has become a matter of some concern.

1.D. The Role of VA Mycorrhizal Fungi in the Recovery of Disturbed Land

Nicolson (1967) suggested that plant growth in industrial wastes could be improved by incorporating VA mycorrhizal fungi. Daft and Nicolson (1974), Daft, Hacskaylo and Nicolson (1975), and Daft and Hacskaylo (1976) observed extensive infection of most plants colonizing coal wastes in Pennsylvania and Scotland and hypothesized that infection was essential for successful colonization by most (but not all) plants. Medve (1977) and Shuffstall and Medve (1979) found that many colonizers of three-year-old bituminous coal mining spoils in Pennsylvania were infected, but that numerous annuals (especially in the Polygonaceae) were not. Extensive infection was not positively correlated with colonization success. Khan (1978) reported similar results for Australian coal spoils, noting that members of the Proteaceae were successful non-mycorrhizal invaders. Plants inhabiting reclaimed surface mines and colonizing mine spoils in arid and semi-arid regions of western North America frequently are infected, though much of the research has been limited to the northern great plains (Christensen and Williams, 1977; Stahl et al., 1979; Allen and Allen, 1980; Call and McKell, 1980; Loree and Williams, 1981).

Inoculation with endophytes and with soil containing endophytes can improve the growth and survival of desirable revegetation species. Williams, Wollum and Aldon (1974) and Aldon (1975) improved the growth of fourwing saltbush (Atriplex canescens (Pursh.) Nutt. by adding endophyte-bearing soil to sterilized soil and mine spoil. Daft et al. (1975) and Daft and Hacskaylo (1976, 1977) found that the growth of red

maple, maize, alfalfa and other plants in mine spoil was improved by introducing endophytes which had been isolated from the spoil areas. Lindsey, Cress and Aldon (1977) saw similar results for rabbitbrush (Chrysothamnus nauseosus (Pall.) Britton) grown in sterilized and unsterilized coal mine spoil. Khan (1981) improved the growth of onions (Allium spp.) in unsterilized spoil by adding pot-culture inoculum of Glomus spp. and Sclerocystis rubiformis. Lambert and Cole (1980) noted improved growth of forage species on mine spoil caused by the addition of 1 cm layers of endophyte bearing topsoil. In popular reviews, Cundell (1977) and Aldon (1978) urged experimental application of these organisms to revegetation efforts.

Many plants may require VA mycorrhizal infection in order to survive on disturbed lands, but most successful pioneers of harsh sites and new soils are probably non-mycorrhizal. Marx (1975) hypothesized that many such plants are successful because they do not require infection, and that succession of disturbed ecosystems might be influenced by the quantity and quality of mycorrhizal inoculum present. Christensen and Williams (1977) noted a prevalence of non-mycorrhizal weedy invaders in mining-disturbed areas. They felt that persistence of communities of these plants would fail to support endophyte populations and thus delay or prevent the influx of mycorrhiza-dependent species. Similar conclusions were reached by Reeves et al. (1979). They hypothesized that:

1. Disturbance of soil leads to reduction and possibly elimination of propagules of mycorrhizal fungi.

2. Reduced numbers of propagules leads to a lower potential for infection of new host plants.
3. Non-mycorrhizal species become established because normally mycorrhizal plants die in the seedling stage (for lack of mycorrhizal fungi).
4. Success of non-mycorrhizal species further reduces the propagules of mycorrhizal fungi since the fungi are obligate symbionts.
5. Total elimination of mycorrhizal fungi obviates competition by mycorrhizal higher plants.
6. Succession is slowed because of the lack of potential mycorrhizal fungi.
7. The harsher the site the greater the potential for elimination of mycorrhizal propagules and, therefore, a longer time is required for re-establishment of mycorrhizal vegetation.

Miller (1979) stated that the mycorrhizal status of plants on disturbed sites is related to the degree of disturbance and to the harshness of the site. He proposed that the ruderal (weedy invasive) reproductive strategy and the non-mycorrhizal condition are related, and that both attributes are essential for colonization of certain severely disturbed sites in harsh environments.

Species vary in their degree of dependency on mycorrhizal endophytes. Janos (1980) discribed a range of ecological dependency characterized by three main types. Non-mycotrophs are not susceptible to mycorrhizal infection and do not require an inoculum source. Many ruderal pioneers of harsh sites (including many Chenopods) are of this

type. Facultative mycotrophs are susceptible to infection but may not require it, especially in relatively fertile environments. Baylis (1975) suggested that grasses may be somewhat independent of mycorrhizae except at low phosphate levels. Non-mycorrhizal mature specimens of Agropyron spp. and other grasses have been observed on reclaimed surface mines in Wyoming (Miller, 1979; Loree and Williams, 1981). Facultative mycotrophy may in part explain the ability of some grasses to colonize bare spoils and other harsh sites. Obligate mycotrophs are those which would rarely occur in nature without endophytes. While the relative dependency of most dryland species has not been evaluated, certain desirable revegetation species (perhaps especially some woody perennials) are likely to require infection for successful establishment. Lindsey and Beavis (1981) found that several dryland shrubs differ in their response to inoculation. Rabbitbush (Chrysothammus nauseosus) and winterfat (Ceratoides lanata Pursh.) were particularly responsive. A large growth response does not necessarly indicate obligate dependency, but differences in response between species is some indication of their requirements. Obligate mycotrophs probably fail to become established in sites of very low inoculum density, and may only do so after endophytes have colonized the area. While there is little concrete evidence to support this hypothesis, obligate mycotrophy may explain some of the significant problems of seedling establishment in disturbed sites. If this is so, then these organisms are determinants of community composition during early succession, and they may in part control the progress of succession. This concept requires much more investigation, but the situation implies

that manipulations of endophyte populations might be used to enhance the revegetation of disturbed land.

1.E. Using VA Mycorrhizal Fungi for Land Reclamation

Ectomycorrhizal fungi and symbiotic bacteria are used in reforestation and revegetation. VA mycorrhizal fungi have not yet been extensively applied in commercial agriculture, forestry, or land reclamation, although commercial development is in progress (Menge, 1981). Development has been hindered by the lack of pure culture methods, but dual cultures with host plants in greenhouses may suffice for large scale inoculum production (Mosse, 1981).

Research oriented toward reclamation should emphasize methods of maintaining inoculum levels in soil (Allen, 1980) as well as techniques for introducing endophytes to disturbed sites.

Sound mining and construction methods can help to ensure mycorrhizal development following disturbance. Most states require that soil be saved and respread after mining (Schuman and Power, 1981). Topsoil storage is minimized at most mines to help retain soil fertility and plant propagule viability. Coal strip mining can eliminate stockpiling altogether by placing soil salvaged from one area onto another. Spoil fields without topsoil are thus somewhat restricted. Nevertheless, extensive areas of orphaned spoils remain unreclaimed, and new expanses of raw spoils are created each year. Alternative energy developments like oil shale and tar sand extraction may add vast areas of inhospitable spoils in harsh environments. The application of proven reclamation procedures (e.g. topsoil amendment) to these areas is encouraged. Even small additions of soil (Lambert and Cole, 1980) and organic mulches (Danielson et al., 1979; Zak and Parkinson, 1981) can

enhance mycorrhization in spoils. It is generally more ecologically sound to preserve the soil microflora than to try to recreate it.

More deliberate manipulations such as inoculation of seeds or seedlings will probably be most applicable to the more extreme situations where natural inoculum is minimal and plant establishment is poor.

Seed inoculation has been successful in experimental agronomic applications (Hattingh and Gerdemann, 1975; Gaunt, 1978), including the reclamation of eroded pasture soils in New Zealand (Hall and Armstrong, 1979; Hall, 1980). The endophytes produce resistant propagules seemingly suitable to inclusion in seed pellets or slurries, but the ability of these propagules to initiate infection under conditions of stress is questionable. Seeding of disturbed lands is the most generally successful revegetation technique, and research into this inoculation method is critically needed.

Inoculation of transplanted seedlings is more feasible at this time. Transplantation is expensive, but can markedly enhance the survival of many species. Inoculation of container-grown shrubs and grasses enhanced their growth and survival in Utah oil shale spoils (Call and McKell, 1980). Evaluation of the commercial practicality of this approach must await further study.

Much of this is speculation. It has not been demonstrated that the use of VA mycorrhizal fungi in revegetation efforts can enhance plant growth on a practical basis. There is even evidence that infection can adversely affect plants (Baylis, 1967; Hays et al., 1981) including those growing in fertilized mine spoils (Kiernan, Hendrix and Maronek,

1981). VA mycorrhizal pathogenesis may be more important than most researchers believe. Serious problems in inoculum production and inoculation technology must be overcome before commercial application can take place. Even if commercial development is feasible, VA mycorrhizal biotechnology will not be a cure-all for the problems of land reclamation. Most important, it is essential for investigators in the field to avoid unsubstantiated generalizations and to adopt a pragmatic and objective approach to mycorrhiza research.

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SECTION 2

COLONIZATION BY WESTERN WHEATGRASS (Agropyron smithii Rydb.)
OF VESICULAR-ARBUSCULAR MYCORRHIZAL FUNGI DURING
THE REVEGETATION OF A DISTURBED SITE

Contents

2.A. Introduction

Vesicular-arbuscular (VA) mycorrhizal infection is extensive in many plant communities of the semi-arid western United States (Gerdemann and Trappe, 1974; Williams and Aldon, 1976; Davidson and Christensen, 1977; Molina, Trappe and Strickler, 1978; Reeves et al., 1979; Miller, 1979; Trappe, 1981). The ubiquity of these organisms and their apparent role in nutrient cycling suggest that they have a powerful functional influence on many dryland ecosystems (Trappe, 1981).

Severe land disturbance caused by the surface mining of energy-related resources can profoundly disrupt plant communities and their associated endophytes. Such disturbances may leave little viable inoculum capable of initiating mycorrhizal infection in plants colonizing the affected areas (Reeves et al., 1979; Miller, 1979). This may influence community composition during early recovery by precluding the establishment of species with high mycorrhizal dependency (Reeves et al., 1979; Janos, 1980). Plants colonizing mine spoils and those introduced during land revegetation frequently become infected (Daft and Nicolson, 1974; Daft, Hacskaylo and Nicolson, 1975; Daft and Hacskaylo, 1976; Khan, 1978; Reeves et al., 1979; Miller, 1979; Ponder, 1979; Allen and Allen, 1980). Levels of infection in communities are thought to increase as recovery progresses (Reeves et al., 1979; Allen and Allen, 1980), but the rate at which colonization occurs is unknown. Knowledge of these rates may help to determine the necessity of artificial manipulation of endophyte populations (by inoculation or other means) in future reclamation efforts. In general, fundamental information regarding the ecological importance of VA mycorrhizal

interactions in semi-arid lands is very limited and is considered to be "urgently needed research" (Trappe, 1981).

The primary objective of this study was to investigate the colonization dynamics of endophytic fungi during the early recovery of revegetated sites at one Wyoming surface mineral mine, as indicated by the mycorrhizal status and the frequency of Endogonaceous spores associated with specimens of Agropyron smithii Rydb. collected from sites of various ages (the time elapsed since initial revegetation). A secondary objective was to study the effects of extractable soil phosphorus concentrations and soil organic matter contents (a potential inoculum source) on the occurrence of infection in seedlings from a newly revegetated site.

2.B. Materials and Methods

2.B.1.Study Sites

The study took place on the revegetated portions of an open-pit uranium mine in the Shirley Basin of southeastern Wyoming (42°20'N, 106°13'W), a primary uranium producing region of the U.S. The area has a rolling topography with a mean elevation of 2150 m. The landscape is a semi-arid short-grass steppe dominated by big sagebrush (Artemisia tridentata Nutt.), with mean annual precipitation of 29 cm and persistent dessicating winds. Local topsoils are primarily shallow clay loams with saturated paste pH circa 7.7, electrical conductivity circa 0.5 mmhos/cm, and organic matter contents of about 2.4% (Rauzi and Tresler, 1978).

Before overburden (spoil) excavation, topsoil is salvaged and stockpiled, to be replaced during post-mining land reclamation. Intervals of topsoil storage often exceeded several years. Prior to the study, a series of plots was reclaimed and seeded from 1973 to 1981, resulting in a chronosequence of sites which were from one month to seven years old at the time of sampling. Topsoil redistribution was somewhat heterogeneous, resulting in surface growth media of variable soil content, sometimes consisting entirely of spoil material. The reestablished plant communities on these sites were dominated by Agropyron spp., other grasses, and native leguminous forbs. Aboveground biomass appeared to be substantially greater in the older sites.

2.B.2. Sampling of plants and soil

Western wheatgrass was selected for intensive sampling because it was dominant on the site and because its mycorrhizal interactions have been previously investigated (Reeves et al., 1979; Miller, 1979; Allen and Allen, 1980; Stahl and Christensen, 1982). Duplicate 300 cm^3 soil cores were collected to a depth of 10 cm adjacent to selected specimens. Sampling to this depth frequently included the entire depth of replaced topsoil. Sims and Coupland (1979) reported that circa 30% of the root biomass of a northern mixed prairie site was in the upper 10 cm of soil. Five samples (located so as to avoid the roots of other species) were taken from each plot and from an adjacent undisturbed site during July of 1980. Other species were arbitrarily sampled for a qualitative overview of the general mycorrhizal status. In June of 1981 (circa four weeks after emergence) forty-one samples were collected from a newly revegetated site so as to evaluate seedlings growing in media of variable topsoil content. All samples were stored at 2°C prior to examination.

2.B.3. Laboratory analysis

Sample cores for root extractions were soaked overnight in .2% Na hexametaphosphate. Rooting densities (length of root per volume of soil) and extent of VA mycorrhizal infection were determined by the method of Ambler and Young (1977), a line intersect technique employing a compound microscope and a large plate-glass counting chamber slide. Total and mycorrhizal rooting densities (L_v) are the mean length (cm) of root in 1 cm^3 of soil (Barley, 1970; Black and Tinker, 1979). The

infected percentage of root length is based on the proportion of 3-4 mm segments containing characteristic endophytic fungal structures.

Endogonaceous spores were extracted from 25 g (dry weight) subsamples by the sucrose flotation-centrifugation method of Allen et al. (1979). Spores were identified on the basis of descriptions by Stahl and Christensen (1982). Spore frequency is reported as mean spore number per g (dry weight) of soil.

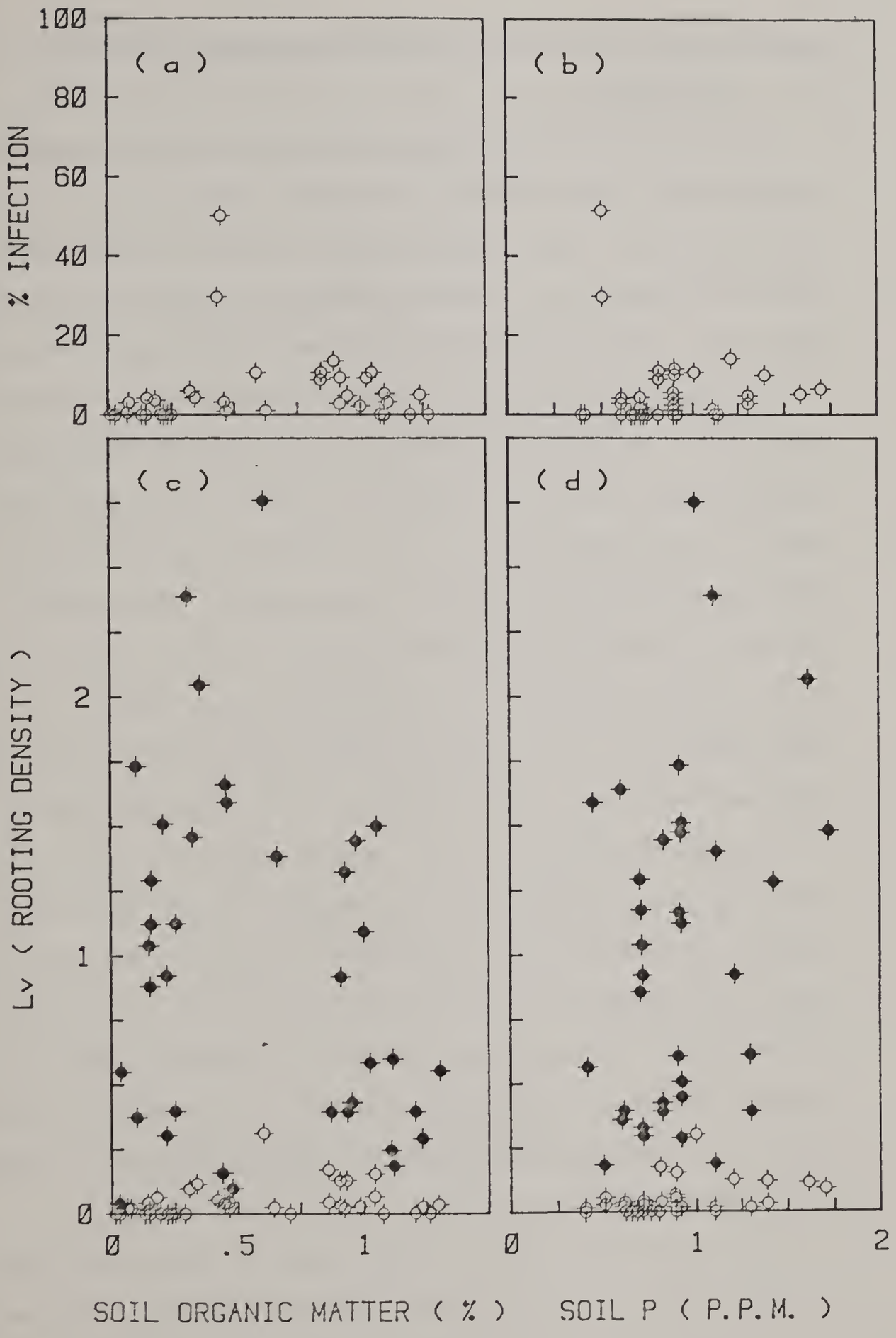
Soil organic matter content (Piper, 1950) was used as an indicator of the proportion of topsoil (vs. overburden material) in the soil cores from the newly revegetated site. $NaHCO_3$ extractable P (Watanabe and Olsen, 1965) was determined for these samples as well.

2.C. Results

2.C.1. Newly revegetated site

Most of the seedlings examined were VA mycorrhizal, but infection levels were very low. Most endophytic mycelia included arbuscules. Vesicles and newly formed spores were infrequent. Linear regression and correlation analysis revealed no significant relationship between soil organic matter content and either total rooting density or level of infection, although specimens from low organic content media were consistently non-mycorrhizal or nearly so (Figure 1). Extractable P content was positively correlated with both total and mycorrhizal rooting density, but was not significantly correlated with percentage infection (Figure 1). Multiple regression analysis further resolved the relationship between total rooting density and the two edaphic factors, revealing this regression equation: Y (total L_v) = .39 - .65 X_1 (% soil organic matter) + 1.07 X_2 (p.p.m. extractable soil P), with a coefficient of determination (R^2) = .27, significant at α = .01. Similar analysis of the combined effect of the two edaphic factors on infected rooting density and on percentage infection did not further resolve these relationships. Infected rooting density was correlated with total rooting density: Y (infected L_v) = -.01 + .05 X (total L_v), with r^2 = .30, significant at α = .01. Infected rooting density was weakly correlated with percentage infection: Y (% infection) = 3.34 + 51.62 X (infected L_v), with r^2 = .12, significant at α = .05.

Figure 1. The effect of selected edaphic factors on rooting density and levels of VA mycorrhizal infection in four-week old Agropyron smithii seedlings from a newly revegetated site. L_v = root length (cm) per cm^3 of soil. Soil P (p.p.m.) = parts per million phosphorus ($NaHCO_3$ extractable). (a) Effect of soil organic matter content on percentage infection. Linear regression equation: Y (% infection) = 4.14 + 2.31 X (% organic matter), with a coefficient of determination (r^2) = .01, not significant at α = .05. (b) Effect of soil P content on percentage infection. Y (% infection) = 7.76 - 2.64 X (p.p.m. P), with r^2 = .07, not significant at α = .05. (c) Effect of soil organic matter content on total (⌖) and mycorrhizal (⊙) rooting density. Y_1 (total L_v) = 1.16 - .37 X (% organic matter), with r^2 = .05, not significant at α = .05. Y_2 (infected L_v) = .03 + .03 X (% organic matter), with r^2 = .03, not significant at α = .05. (d) Effect of soil P content on total (⌖) and mycorrhizal (⊙) rooting density. Y_1 (total L_v) = .27 + .78 X (p.p.m. P), with r^2 = .13, significant at α = .05. Y_2 (infected L_v) = -.02 + .07 X (p.p.m. P), with r^2 = .12, significant at α = .05.


(a)
(b)
(c)
(d)
% INFECTION
100
80
60
40
20
0
Lv (ROOTING DENSITY)
2
1
0
0
.5
1
0
1
2
SOIL ORGANIC MATTER (%)
SOIL P (P.P.M.)

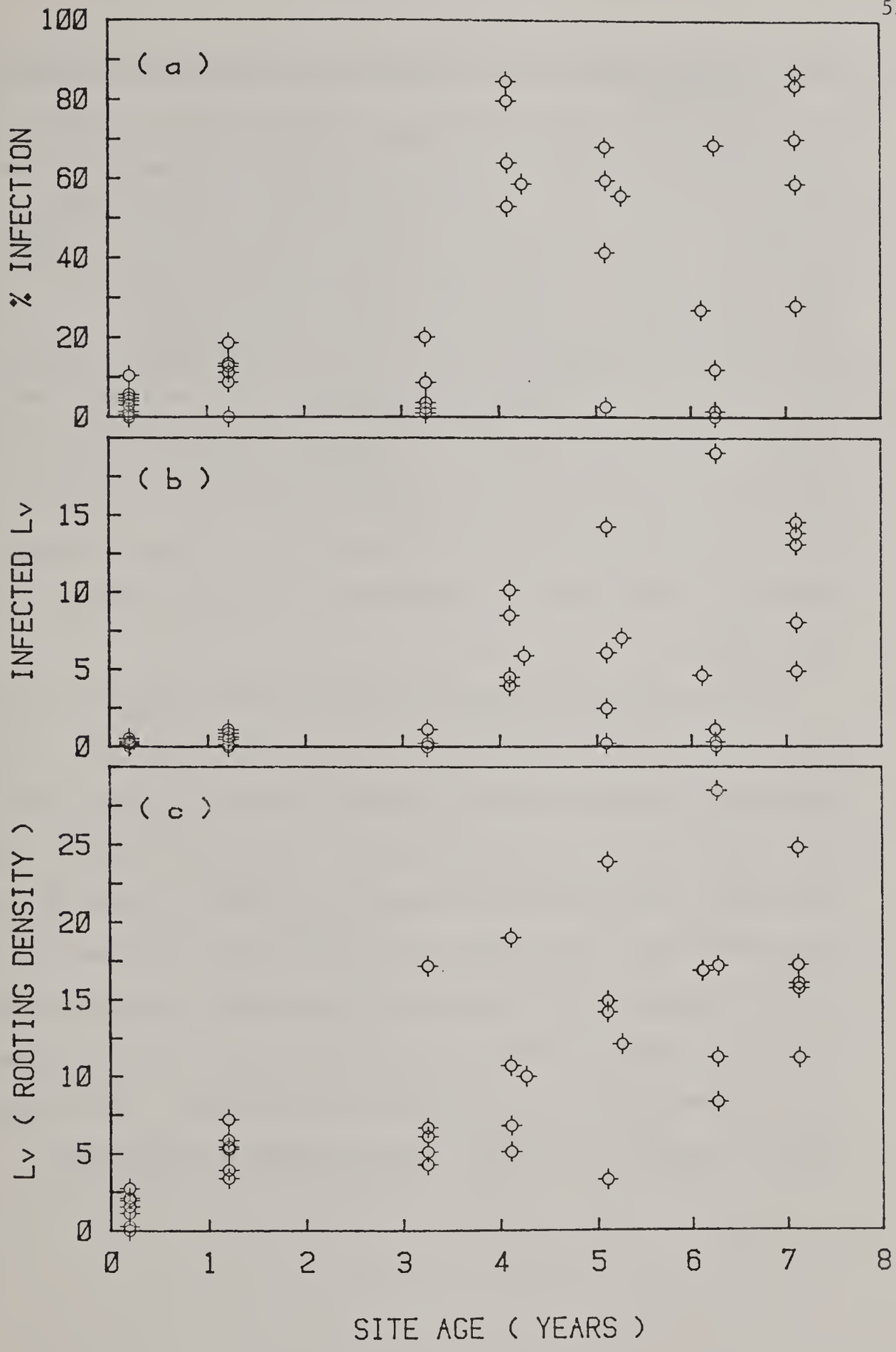
2.C.2. Effect of site age on rooting density and mycorrhizal development

VA mycorrhizal infection was present in sites of all ages and was extensive (but highly variable) in the older sites and in the adjacent undisturbed site. Total rooting density, infected rooting density, and percentage infection all increased markedly as a function of site age (Figure 2). The data exhibited heterogeneous variance and was subjected to $\log_e$ transformation prior to regression and correlation analysis. (Figure 2). Infected rooting density was strongly correlated with total rooting density: $\log_e$ Y (infected L_v) = -3.49 + 1.66 $\log_e$ X (total L_v), with r^2 = .52, significant at α = .01. Multiple regression analysis of the relationship between infected rooting density, total rooting density, and site age revealed this equation: $\log_e$ Y (infected L_v) = -3.45 + .35 X_1 (site age) + .97 $\log_e$ X_2 (total L_v), with R^2 = .56, significant at α = .01. Infected rooting density was correlated with percentage infection: Y (% infection) = .03 + .08 X (infected L_v), with r^2 = .40, significant at α = .01. The intensity of infection in individual root fragments was not quantified, but also appeared to increase with site age. For the sites up to and including those about three years old there was a relatively low proportion of mycorrhizal rooting density. Infection was clustered in localized areas of the younger sites, and many uninfected specimens of ordinarly mycorrhizal species (e.g. Melilotus officinalis L., Medicago sativa L, and several spp. of Agropyron) were observed.

Data from the adjacent undisturbed area was comparable to that from the older sites. Mean total rooting density (L_v) was 22.07 cm per cm^3

of soil, with a standard deviation (s) of 12.89 (n = 5). Mean infected L_v was 10.57 cm per cm^3 of soil, with s = 5.44 (n = 5). Mean percentage infection was 50.7, with s = 16.8 (n = 5).

Figure 2. The effect of site age (time since initial revegetation) on rooting density (L_v = length (cm) of root per cm^3 of soil) and VA mycorrhizal infection of Agropyron smithii growing on the revegetated areas of a surface mine. (a) Effect of site age on percentage infection. Regression equation: Log_e Y (% infection) = -3.46 + .34 X (site age), with a coefficient of determination (r^2) = .22, significant at α = .01. (b) Effect of site age on infected rooting density: Log_e Y (infected L_v) = -.287 + .70 X (site age), with r^2 = .50, significant at α = .01. (c) Effect of site age on total rooting density: Log_e Y (total L_v) = .60 + .35 X (site age), with r^2 = .69, significant at α = .01.

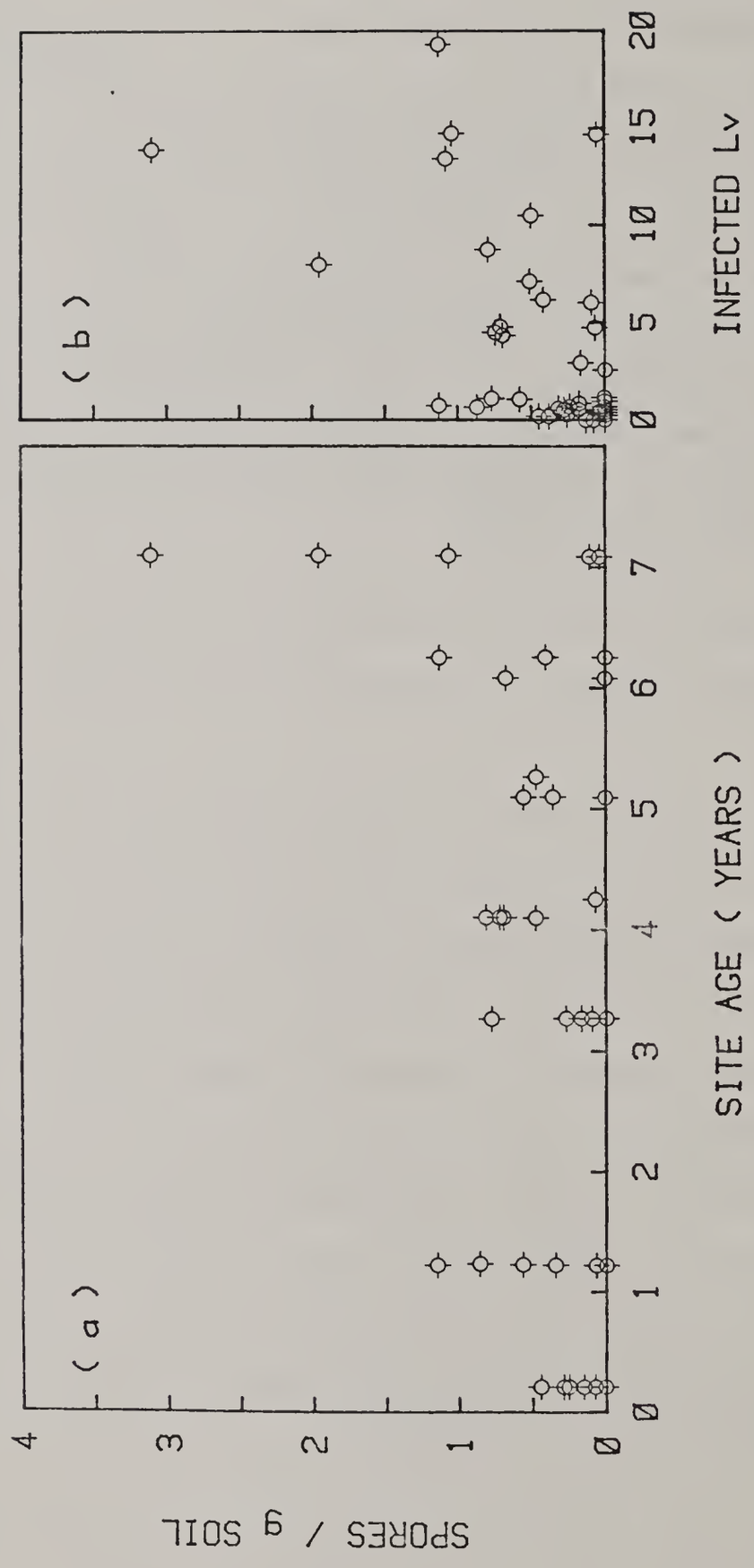

(a)
% INFECTION
100
80
60
40
20
0
(b)
INFECTED Lv
15
10
5
0
(c)
Lv (ROOTING DENSITY)
25
20
15
10
5
0
0
1
2
3
4
5
6
7
8
SITE AGE (YEARS)

2.C.3. Effect of site age and mycorrhizal rooting density on spore frequency

Endogonaceous spores were present in most samples from disturbed sites of all ages and in all samples from the undisturbed site. Propagules of four species were observed. They were (in decreasing order of frequency): Glomus fasciculatus (Thaxter sensu Gerd.) Gerd and Trappe, G. macrocapus Tul. and Tul. var. macrocarpus, G. microcarpus Tul. and Tul., and Entrophospora infrequens (Hall) Ames and Schneider (1979). Spores and sporocarps of G. mosseae (Nicolson and Gerd.) Gerd. and Trappe were observed on the undisturbed site only; no sporocarps of any sort were recovered from the disturbed area. No characteristic examples of either spores or mycorrhizae of the "fine endophyte" were encountered.

Spore frequency was positively correlated with site age, but was more strongly correlated with mycorrhizal rooting density (Table 3). Surprisingly, multiple regression analysis failed to further resolve the relationship between these three factors. The variety of spore types observed per sample seemed to increase as a function of site age, but not significantly. Many spores in the younger sites appeared to be residual propagules which had physically survived the soil stockpiling. It was thus impossible to discern cause from effect in the relationship between spore frequency and mycorrhizal proliferation. Most endophytic mycelia appeared to be either G. Fasciculatus or G. macrocarpus.

Figure 3. The effect of site age (time since initial revegetation) and mycorrhizal rooting density (L_v = length of root (cm) per cm^3 of soil) on the frequency of Endogonaceous spores associated with Agropyron smithii growing on the revegetated areas of a surface mine. (a) Effect of site age on spore frequency. Regression equation: Y (spores/g soil) = .13 + .10 X (site age), with a coefficient of determination (r^2) = .16, significant at α = .05 (b) Effect of infected rooting density on spore frequency = Y (spores/g soil) = .20 + .07 X (infected L_v), with r^2 = .34, significant at α = .01.


SPORES / g SOIL
4
3
2
1
0
(a)
0 1 2 3 4 5 6 7
SITE AGE (YEARS)
(b)
0 5 10 15 20
INFECTED Lv

2.D. Discussion

This investigation supports the findings of earlier research (Reeves et al., 1979; Miller, 1979; Allen and Allen, 1980) regarding the colonization of severely disturbed semi-arid land by vesicular-arbuscular mycorrhizal fungi. It revealed a progressive increase in the magnitude of the endophyte community following a post-disturbance interval of limited mycorrhizal proliferation which lasted several years. While the cause of this interval is not entirely clear, low inoculum density may have been partly responsible. Topsoil replaced during reclamation was the most obvious source of Endogonaceous inoculum, though other mechanisms (such as wind dispersal) cannot be discounted. It was hypothesized that mycorrhizal development in seedlings would be related to the proportion of topsoil in the growth media. It appeared that very small quantities of soil-borne inoculum could initiate infection. Ponder (1979) observed mycorrhization of seedlings inhabiting mine spoil without topsoil, implying that low inoculum densities had been effective. Previous studies (Daft and Nicolson, 1969; Moorman and Reeves, 1979) have indicated that low levels of inoculum can initiate infection, but that it progresses slowly. In this study, higher soil contents in the media did not necessarily produce more infection; topsoil salvage and replacement does not guarantee mycorrhizal development, particularly when the soil is stockpiled for prolonged intervals. However, VA mycorrhizal development can be enhanced by applications of topsoil (Lambert and Cole, 1980) while mycorrhizal development in spoils without added soil may be comparatively slow (Zak and Parkinson, unpublished). Soil storage

intervals should be minimized to lessen inoculum depletion (Rives et al., 1980).

Endophyte development could feasibly be inhibited by phosphate fertilization during revegetation, but the minimal fertilization of this study site resulted in low levels of extractable P which were apparently insufficient to do so. Instead, mycorrhizal rooting density was positively correlated to extractable P content. Danielson, Zak, and Parkinson (1979) noted greater mycorrhizal root length of A. trachycaulum (Link) Malte. in fertilized mine spoil than in comparable unfertilized spoil. Higher levels of P may cause lower percentage infections in some cases, but not necessarily lower levels of infected biomass. Information on the threshold levels of P necessary to inhibit infection in reclaimed sites would be useful.

Time is clearly a factor in the reestablishment of symbiont communities following disturbance. The rates observed herein are comparable to those seen by Allen and Allen (1980), who found that percentage infection levels in revegetated Wyoming sites had increased to roughly 50% of those in undisturbed prairie within 2-3 years. They did not discuss the amount of root material involved. Zak and Parkinson (unpublished) observed significant increases in infected root length after four years of A. trachycaulum growth in unamended coal spoil. These rates are in marked contrast to mycorrhizal development rates in undisturbed soil. Schwab and Reeves (unpublished) reported 80% infection of four-week old A. smithii seedlings grown in fresh topsoil in a greenhouse.

Elapsed time, certain soil characteristics, and site reclamation practices helped to determine the rate of mycorrhizal development. Miller (1979) stated that the severity of disturbance and the harshness of the site control the occurrence of VA mycorrhizae in such situations, while Allen and Allen (1980) felt that inoculum density, edaphic characteristics, plant cover, host genotype and elapsed time are critical determinants as well. Danielson et al., (1979) found that inorganic fertilization and organic amendment could modify development rates in mine spoil. It is apparent that VA mycorrhizal development in disturbed areas is controlled by a complex of interacting factors which are not yet fully understood.

The occurrence of non-mycorrhizal specimens of ordinally infected species was also noted by Miller (1979), who observed mature non-mycorrhizal A. smithii on a severely disturbed Wyoming site. Although VA mycorrhizae are generally prevalent in grasslands, many grasses are apparently "facultative mycotrophs" which may not be obligately dependent on their endophytes (Baylis, 1975; Janos, 1980). This could account in part for the success of A. smithii as a revegetation species for harsh sites.

In sites less than four years old, the great majority of root material was nonmycorrhizal. Since the extracortial mycelia of established mycorrhizae may be a primary inoculum source for seedlings (Read, Koucheki, and Hodgson, 1976), low mycorrhizal rooting densities could inhibit the establishment of seedlings of highly dependent species (Reeves et al., 1979), especially if the residual spore inoculum has been depleted. Any such inhibition at this study area would have been

alleviated eventually by endophyte colonization, but could have contributed to seedling failures during initial revegetation. This would depend on the inoculum level required to satisfactorally initiate symbiosis and on the degree of host dependency during the seedling stage. Further investigation of these characteristics of desirable revegetation species is needed.

Land reclamation science stands to benefit substantially from VA mycorrhizal research. Seedling establishment performance could possibly be improved by selecting species suitable to the mycorrhizal infection potential (sensu Reeves) of a site. Ecologically sound mining methods (e.g. prompt topsoil replacement) should enhance the recovery of symbiotic interactions. Endophyte inoculation methods could be particularly applicable to situations where little soil is involved, such as tailings piles, bare spoil, or spent oil shale.

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CHAPTER II

DORMANCY, GERMINATION, EMERGENCE, AND ECOLOGY OF WOODY SHRUB SEEDS IMPORTANT FOR ARID AND SEMI-ARID LAND RECLAMATION IN THE WEST

With Special Attention to Gardner Saltbush (*Atriplex gardneri*)

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INTRODUCTION

Shrubs play an important role in many western arid and semi-arid rangeland ecosystems. They provide valuable winter browse for domestic and wildlife species, and provide protective, mating, nesting, and other aspects of wildlife habitat (McKell 1975). Arid land shrubs generally are more adapted and grow better than perennial grasses and forbs in areas of high drought, salinity and alkalinity stresses (McArthur et al. 1974). This feature enables shrubs to contribute substantially to arid land ecosystem functioning by providing soil stabilization (in some desert shrubs, nearly 80% of total biomass is devoted to underground biomass), nutrient pools for associated plants, snow catchments for watershed development, and overall microenvironment development (Caldwell 1974, McKell 1975, McArthur 1981).

Shrub reclamation in arid and semi-arid lands

Unfortunately, shrubs have largely been neglected for use in strip-mine reclamation when compared to the use of grasses and forbs (Meyn et al. 1975). There are several reasons for this: (1) the emphasis of past reclamation research has been on seeding grasses because most reclamation in the 1970's was conducted in the Northern Great Plains, an area dominated vegetatively by temperate grass species, forbs and mesophytic shrubs (Packer 1974, Richardson et al. 1975); (2) shrub seed sources are unreliable and expensive for some species, and non-existent for many; (3) seedings with native shrubs have generally

failed (Bleak et al. 1965, Sindelar et al. 1974, Plummer 1976, McKell et al. 1979, DePuit and Coenenberg 1980).

Seeding failures of shrubs have occurred because:

1) shrub seed quality is often low with poor seed fill and viability. Often, within a species, extremes in seed fill and viability occur from host plants growing adjacent to one another or from year to year on the same host plant.
2) shrub seed is often difficult to germinate due to dormancy factors (Malcolm 1972, Gerard 1978, Van Epps and McKell 1980). Seed dormancy is especially complex in native shrubs. These species have generally evolved in harsh environments where the adaptive features of dormancy are enhanced to insure that germination will occur when only the most ideal conditions exist for seedling survival.
3) seedlings germinated from non-adapted seed originating on a site dissimilar to the disturbed area (commercial seed).
4) seedlings died due to poor seedling vigor, herbivore predation, poor seedbed preparation, wrong time of seedling, and other factors (Springfield 1970).

Objectives of this study

This study was initiated to determine methods of enhancing germination and establishment of native and introduced woody shrubs for use on disturbed lands. The study concentrated on three broad objectives:

1) to characterize dormancy and germination in seeds of woody shrubs important for arid and semi-arid land reclamation, and

to develop treatments which will overcome seed dormancy and enhance germination,

2) to enhance field establishment by seeding of a woody shrub species through the use of seed treatments developed in objective 1, and

3) to characterize some aspects of the ecology of seeds of a particular woody shrub species important for arid land reclamation in Wyoming.

This report is comprised of 3 major sections, each corresponding to a study objective listed above:

Section 1- Characterization of Dormancy and Laboratory Germination in Seeds of Woody Shrubs Important for Arid Land Reclamation

Section 2- Field Establishment of Gardner Saltbush by Seeding

Section 3- The Ecology of Gardner Saltbush Seeds and Seeds of Related Species.

Species evaluated in this study

Emphasis in the study has been on one species, Gardner saltbush (Atriplex gardneri (Moq.) D. Dietr.). Dormancy and laboratory germination (Section 1), field emergence in response to seed treatment (Section 2), and numerous aspects of this species' seed ecology (Section 3) have been studied.

Some studies regarding dormancy and laboratory germination of big sagebrush (Artemisia tridentata Nutt.), green and rubber Rabbitbrush (Chrysothammus spp.), and Siberian peashrub (Caragana arborescens Lam.)

have been conducted, although no in depth work was performed on these species. These studies are outlined in Section 1.

Financial support

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SECTION 1

CHARACTERIZATION OF DORMANCY AND LABORATORY GERMINATION IN SEEDS OF WOODY SHRUBS IMPORTANT FOR ARID AND SEMI-ARID LAND RECLAMATION

Contents

1.A. Introduction

1.A.1. Seed dormancy in woody shrubs

The literature describes seed dormancy as being either seedcoat related (physical dormancy) or embryo related (embryo, physiological or "true" dormancy) (Copeland 1976, Khan, 1977). Seedcoat dormancy may be due to chemical inhibitors in the seedcoat (or bract surrounding the seedcoat), or due to impermeability of the seedcoat to gases (particularly oxygen) and/or water. Young and Evans (1981) and Nord (1956) have shown that dormancy in some native woody shrub species like bitterbrush (Purshia tridentata) can be attributed almost entirely to the impermeability of the seedcoat to oxygen, thus inhibiting respiration by the embryo. Mechanical resistance of the growing embryo by the seedcoat has been described as another kind of seedcoat dormancy, but recent research has indicated that this may not be as important a factor as was previously thought (Mayer and Marbach 1981). Embryo dormancy occurs when seeds fail to germinate because of physiological immaturity of the embryo even though morphological growth is complete (Copeland 1976). Alleviation of embryonic dormancy by natural physiological maturing of the embryo through time or by environmentally induced means (stratification, light, temperature, humidity, etc.) is called after-ripening (Khan 1977).

Although more frequent in a few families, seed dormancy shows no direct relationship to taxonomy (USDA 1974). It does seem to be especially prevalent in woody species. Seeds of woody plants tend to have deep embryo dormancies as well as tough, thick and/or hard seedcoats and/or accessory structures (Young and Evans 1981). In

addition, woody species have generally been neglected for domestication which, in time, tends to reduce seed dormancy in a species (Copeland 1976).

1.A.2. Evaluating dormancy in woody shrub seeds

Attempts were made in this study to characterize dormancy in a particular species as being either seedcoat related, embryo related, or both. Generally, known dormancy breaking and germination enhancement methods were used to classify types of dormancy.

1.B. Gardner Saltbush Dormancy and Germination

1.B.1. Introduction

Members of Chenopodiaceae including Atriplex species play an important role in salt desert shrub zones. Not only are Atriplex species adapted for growth in these harsh zones of saline/alkaline soils and aridity, they are also very important for soil erosion control and wildlife habitat. Many Atriplex species furnish nutritious and preferred browse for wildlife and domestic livestock (Springfield 1970). Caldwell (1974) noted that Atriplex species are able to maintain reasonably high tissue nitrogen levels despite very low available soil nitrogen concentrations.

Gardner saltbush (Atriplex gardneri) is a low growing, woody half shrub (20-50 cm tall) which occurs in relative abundance in salt desert shrub ecosystems of the Intermountain west. The forage value (in terms of production, palatability and nutrition) of Gardner saltbush is not as great as Fourwing saltbrush (Atriplex canescens), probably the most important of the Atriplex species. However, Gardner saltbush does

provide valuable winter browse and may be better adapted than Fourwing saltbrush to extremely arid conditions, severe winter conditions, high alkalinity and salinity, and finer textured soils (Nord et al. 1971, Welch 1981, Vories 1981, Stubbendieck et al. 1981).

Gardner saltbush is closely related to Nuttall saltbush (Atriplex nuttallii S. Wats.) and in some publications is considered a subspecies of Nuttall saltbush (Atriplex nuttallii gardneri (Moq.) Hull and Clements). Stubbendieck et al. (1981) recognizes Gardner saltbush as a distinct species (Atriplex gardneri (Moq.) D. Dietr.). Gardner and Nuttall saltbush occur in separate geographic regions (Gardner saltbush is found throughout Wyoming, Utah, Eastern Nevada, Southeastern Idaho, and Western Colorado, while Nuttall saltbush occurs in more northern regions of Idaho, Montana, and the Dakotas) and this may in fact be the only major difference between the two species (subspecies) (Stubbendieck et al. 1981).

For the purpose of this study, literature concerning both species was incorporated into development of seed treatments, laboratory procedures, field plantings and data analysis. However, it is recognized that seed response to treatment and growing conditions could be different for each species, especially due to their apparent regionality.

1.B.2. Methods and Materials

1.B.2.a. Seed procurement. Commercial sources of Gardner saltbush seeds are few, rather unreliable (there is a great deal of confusion over the taxonomy of this species as mentioned above), and usually not regionally compatible with the Wyoming environments where we chose to

study emergence and establishment. Therefore all seed used in this study was hand collected.

Gardner saltbush seeds were collected in August 1980, 1981 and 1982 from three distinct, regionally isolated populations ranging 15-70 km west of Rawlins in the Red Desert Basin of South Central Wyoming (Figure 1). Each collection site ranged from 3-5 km north of Interstate 80. The three seed populations were called "Knobs," "Rasmussen," and "Red Desert," named after the I-80 exit closest to them. Detailed information about each collection site is found in Section 3 and Appendix A and B. Only seed from the "Knobs" source will be discussed in this chapter.

The steps of Gardner saltbush seed procurement are outlined in Figure 2. Some highlights of this outline are as follows:

(1) Collection - Collection was by hand stripping. The inflorescences of Gardner saltbush are decumbent, making collection by another method inefficient (Eddleman 1978). Eddleman (1978) found the optimum collection period for Nuttall saltbush in Montana was early October. However, we noticed Gardner saltbush seeds in South Central Wyoming were beginning to shed from their stems in August (1980). Therefore, our harvest dates (1980-1982) were during the first two weeks of August.

(2-4) Drying and Cleaning - freshly harvested seeds were spread on a canvas tarp in a warm dry area (Young et al. 1978) to air dry. Large impurities (stems, leaves) were removed via screening, and small impurities (dust, chaff, broken seed parts) were removed using a "Dakota" adjustable plexiglass column blower (Young et al. 1978, p. 18).

(5-6) Size and Weight Grade Separation - Due to the extreme variability in seed size and weight, Gardner saltbush seeds were separated into "Grades" based on these parameters. Grade separation could alleviate much of the variability in seed response to treatment, a common problem in testing native seeds. Metal mesh screens were used to separate seed into 3 size grades, "1," "2," and "3," with "1" being the largest. Size grade "1" seeds were too large to pass screen Size #1 (5 mm). Size grade "2" seeds passed screen #1 but not screen #2

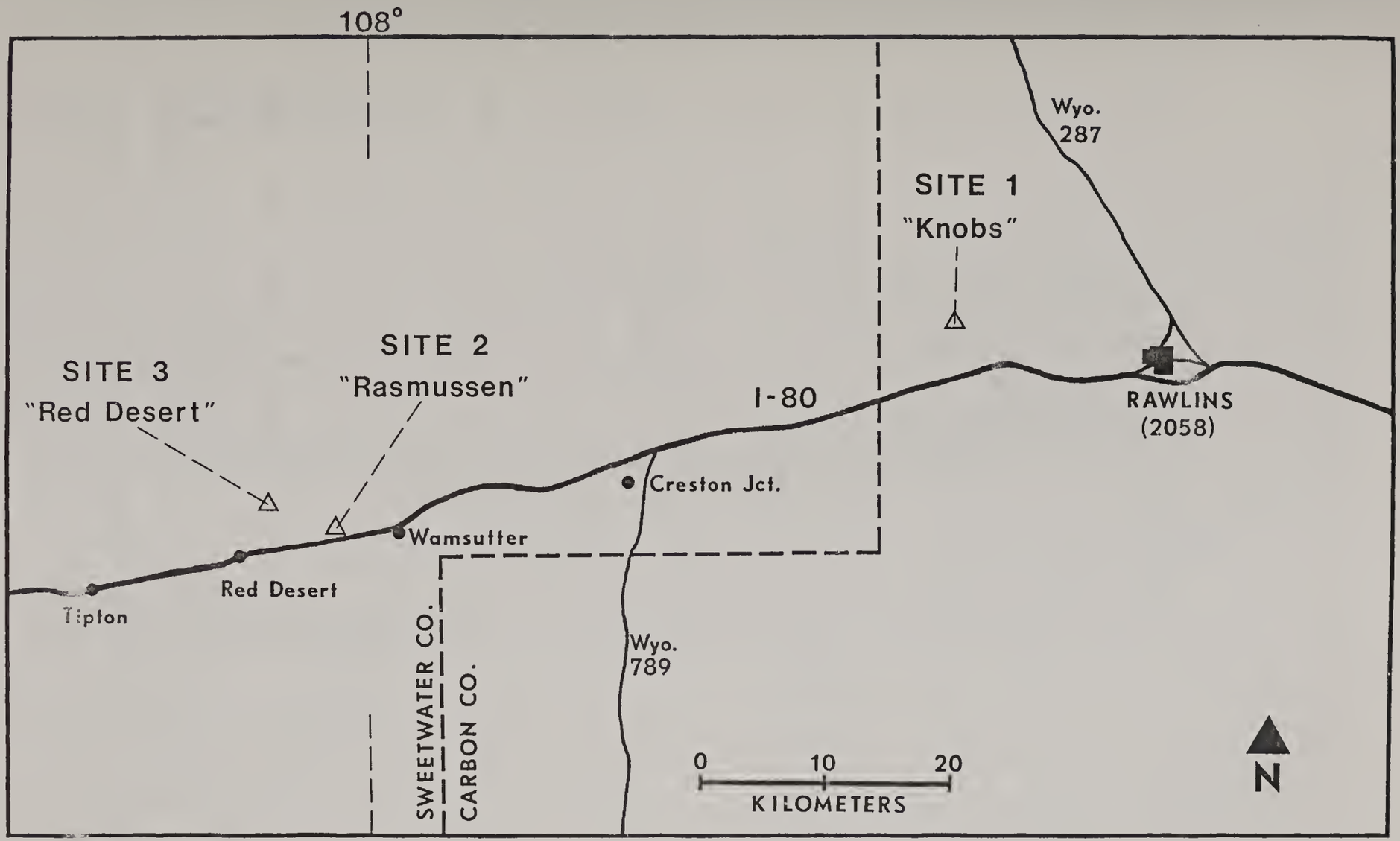

Figure 1. Atriplex gardneri seed collection sites in the Red Desert Basin, South Central Wyoming, August 1980, 1981 and 1982.

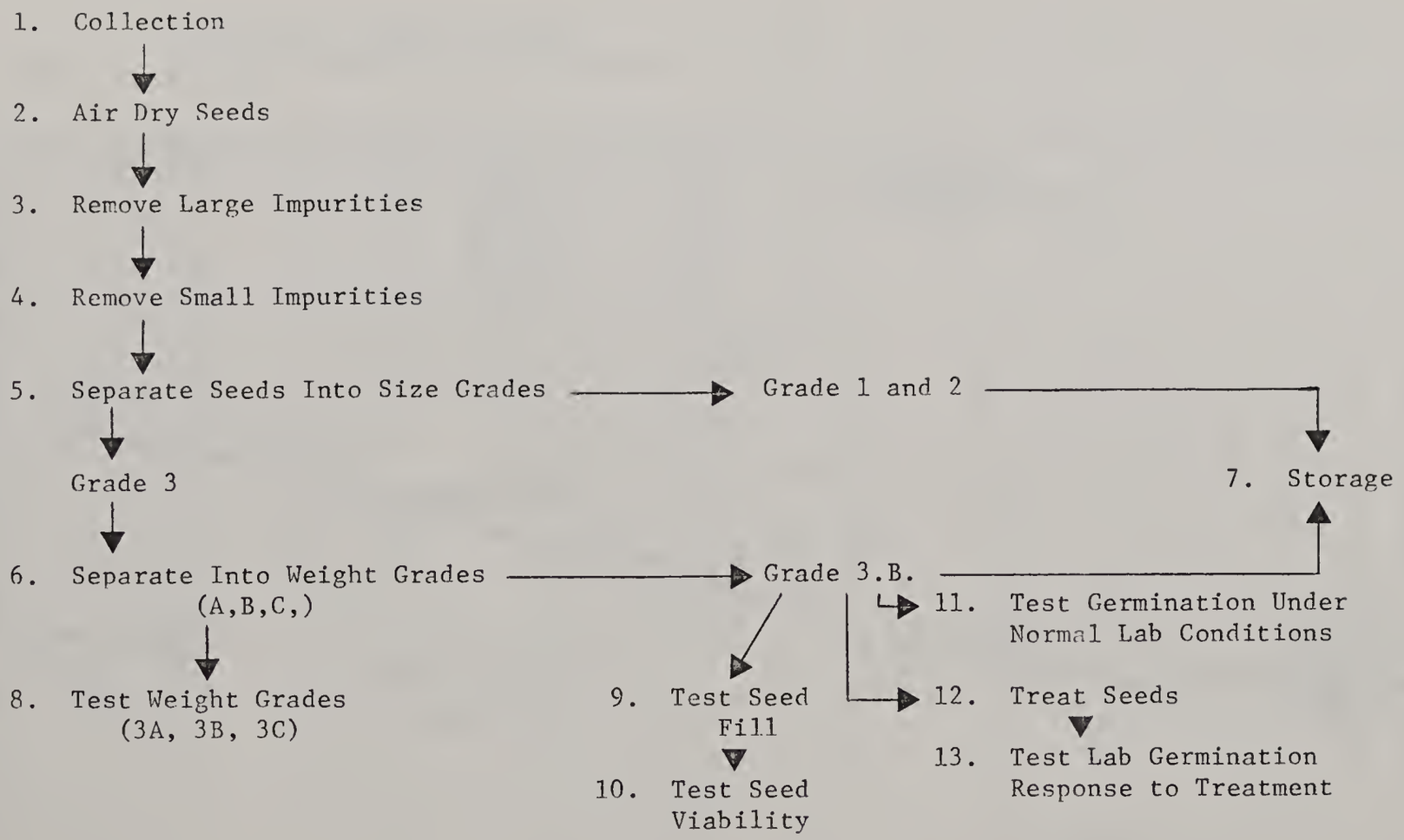

Figure 2. Flow diagram depicting seed procurement procedures of Gardner saltbush seed in this study.

(3 mm). Size grade "3" seed passed screen #2. Size grade "3" seeds were used for further experimentation because grades "1" and "2" did not yield sufficient seed quantities.

Size grade "3" seeds were separated using a "Dakota" column blower into 3 weight classes, "A," "B," and "C," with "A" being the heaviest seeds. Weight class "C" seeds passed up the column with the blower aperture arbitrarily set on 20 . Although this yielded the greatest volume of materials, most of it was empty utricles and broken seed parts. Weight class "B" seeds were too heavy to pass up the column at a blower setting of 20 but passed with the blower set on 30. Weight class "A" seeds were too heavy to pass the column at a blower setting of 30 (as the aperture at the top of the Dakota blower increases, more air pressure flows through the column and heavier seeds can be lifted. A trap at the top of the column catches lifted seed). Weight class "A" yielded few seeds. Therefore weight class "B" seeds were used for most of the experimentation.

(7) Seed Storage - Seeds were stored in dry paper bags at 20°C. Springfield (1970) found that fourwing saltbrush seeds maintained viability for 6 years under these conditions. Foiles (1974) recommended storing Nuttall saltbush seeds in cloth bags at room temperature.

1.B.2.b. Fill and viability testing. The female Gardner saltbush flower has no perianth, protection being provided by two bracteoles 3-8 mm long which enclose the seed forming a false fruit or utricle (Figure 3) (Young et al. 1980, Stubbendieck et al. 1981). In our study the utricle was considered the "seed" which corresponds to other researchers methods (Nord et al. 1971, Young et al. 1980). There are problems with this generalization, however. Some Atriplex species have two types of seeds beneath the bracteoles, a small, hard black seed and a large, soft brown seed. These two seed types have marked differences in their ability to absorb water, length of viability, and response to seedcoat treatments such as scarification (Osmond et al. 1980, p. 161). Unfortunately the bracteoles surrounding Gardner saltbush seeds are so tightly fused it was impossible to determine accurately whether seeds of

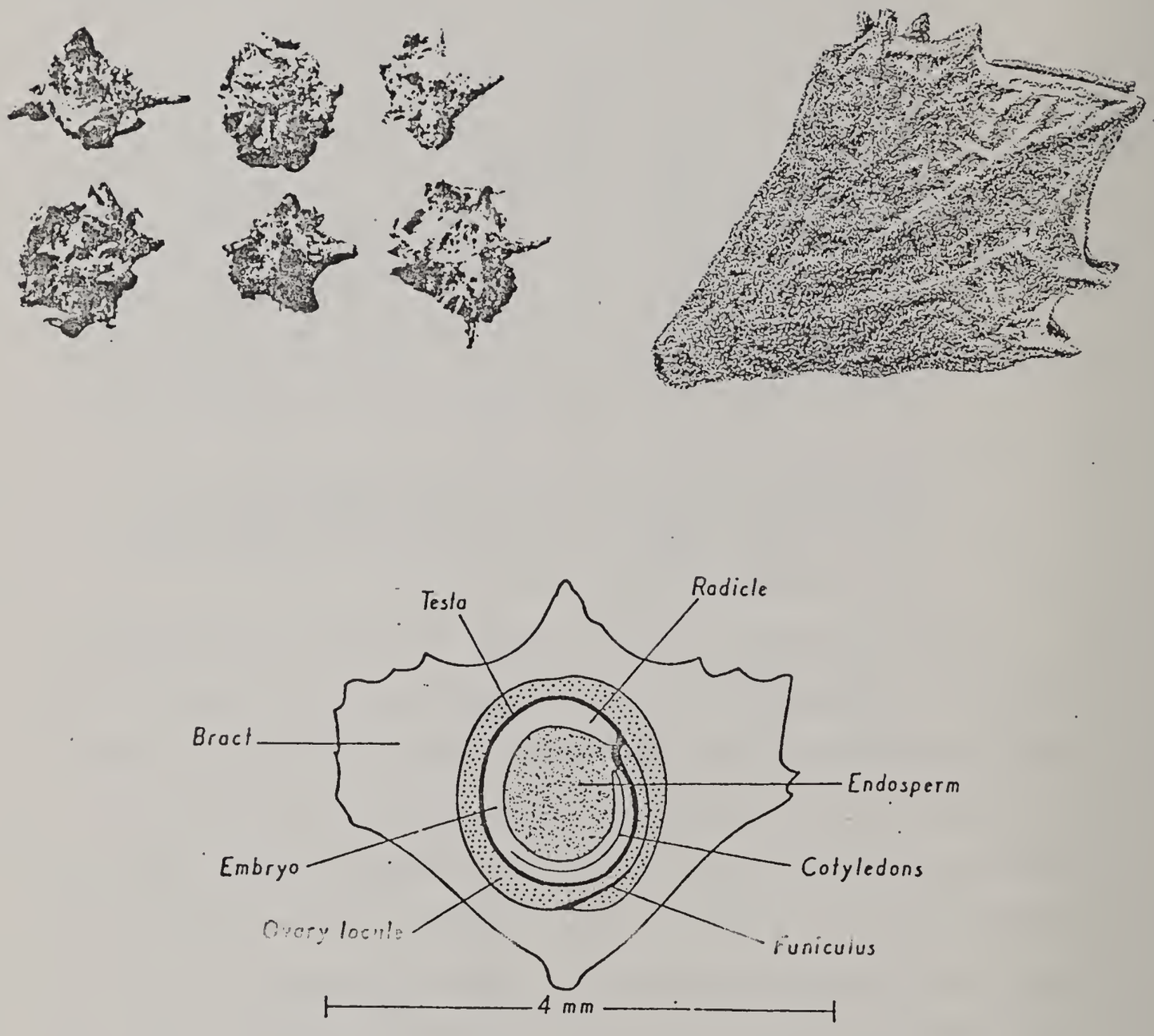

Figure 3. External and internal morphology of typical Atriplex spp. utricles. Lower illustration taken from Lailhacar-Kind and Laude (1975).

this Atriplex species had the dimorphism. It is suspected the majority of Gardner saltbush seeds are of the soft, brown variety, however.

Seed fill was determined by slicing the utricle with a razor blade and observing whether the utricle contained an embryo. The embryos bisected during the seed fill test were then placed in a 0.1 percent 2,3,5-triphenyl-2H-tetrazolium chloride (TZ) solution for 4-8 hours to determine seed viability (Grabe 1970, Springfield 1970, Weber and Wiesner 1980). In the presence of TZ, living tissue can be separated from nonliving or weak tissue. Dormant seeds will stain red if they are viable which gives an estimate of potential germination.

The average percent fill of Knobs (1980) seeds was 60 percent. TZ staining was generally slow and non-uniform. Portions of the embryo (radicle, hypocotyl, cotyledons) stained dark red, pink or not at all. Generally about 90% of the embryos tested in the Knobs (1980) population had some staining and were classified as being viable. Thus, about (.90 x .60) 54 percent of total Knobs 3B seeds were viable.

1.B.2.c. Seed treatments used. We began this study with the assumption that dormancy in Gardner saltbush seeds was related to either the embryo, the seedcoat or both. We exposed seeds to several treatments with the idea that these treatments would enhance germination and also enlighten us as to what kind of dormancy was present in the seeds. Treatments used were divided into 2 major categories: (1) Environmental simulation, and (2) Secondary germination enhancement (Young et al. 1981).

Environmental simulation treatments include those treatments which may occur one way or another in the natural environment of the seed.

Factors to consider are (1) dry storage time, (2) prechill (stratification), (3) washing or leaching of the seedcoat and/or embryo, (4) scarification and (5) light. Each factor will be considered briefly here:

(1) Dry Storage Time - Many seeds will not germinate at the time of seed maturity and dispersal due usually to physiological immaturity of the embryo. Often, a period of time in dry storage allows the embryo to mature physiologically and become ready to germinate. As mentioned in Section I.A.1. of this chapter, in addition to time, prechill, light, temperature, humidity, etc. can affect embryo maturation and all of these processes are collectively called "afterripening." In our study, the term afterripening refers only to the effects of time on embryo maturation. The USDA Forest Service (1974) notes that afterripening in Nuttall saltbush requires 3 months.

(2) Prechill (Stratification) - Many Atriplex spp. seeds require a period of cold moist treatment before they will germinate. Eddleman (1978) recommended Nuttall saltbush seeds be prechilled for 1-2 months at 4°C. In our own tests, we found that Gardner saltbush radicles began to emerge in prechilled seeds (2°C) after 3-4 weeks in cold storage. We assumed that because of this, the required prechill period for Gardner saltbush was near 3 weeks (at 2°C).

(3) Washing or Leaching - Salts and chemicals in the bracteoles, seedcoat and/or embryo may inhibit germination. Washing seeds in running water or soaking them has been shown to remove some of these inhibitors and enhance germination (Beadle 1952).

(4) Scarification - Gardner saltbush has a heavy bracteoles surrounding the seed which may inhibit permeability and radicle emergence (see Figure 3). The presence of salts and chemicals in the seedcoat and bracteole may inhibit germination. Scarification may alleviate these problems. Incidentally, Osmond et al. (1980) noted that in Atriplex spp. with dimorphic seeds scarification stimulated germination in small black seeds but not the large soft brown seeds.

(5) Light - The control of germination by light is readily demonstrated in many seeds. Light may regulate germination in some Atriplex spp. but the literature does not show this to be the case for Gardner or Nuttall saltbush (Eddleman 1978, Vories 1981). Beadle (1952) dismissed light as a factor in the control of Atriplex spp. seed germination under field conditions. Thus, the effects of light were not studied in this project.

Gardner saltbush seeds were exposed to a series of environmental simulation treatments involving dry storage time, prechill, washing, and scarification. Prechill, washing, and scarification were arranged factorially in a Completely Randomized Design to determine main effects of these factors as well as interactions among factors on germination (Table 1). A control and 2 treatments were included within the factor prechill. A control and 2 treatments were included within the factor washing. A control and one treatment were included within the factor scarification. This factorial arrangement produced a total of 18 treatments, 9 of which were scarified and 9 unscarified. Each treatment was replicated six times with 100 seeds per replication. The experiment was conducted at 2, 5 and 15 months after seed harvest so effects of dry storage time and how this parameter might interact with other treatments could be studied.

Seeds were scarified for 20 seconds using a Forsberg scarifier. It was determined that additional scarification time began to damage embryos. Prechill involved maintenance of imbibed seeds at 2°C for 2 or 4 weeks. Wash treatments consisted of exposing seeds to cold running tap water for 1 or 24 hours. Washed seeds were then dried 24 hours before being placed in germination conditions. For the afterripening test, seeds were stored at 20°-25°C in paper bags.

The environmental criteria listed above essentially activate a series of biochemical and hormonal events within a seed and the process of germination begins. Exogenous application of chemicals and/or hormones to imbibed seeds may either replace the required environmental stimulus for germination or, may act synergistically with the

Table 1. 3 x 3 x 2 factorial arrangement of simulated environmental seed treatments to characterize dormancy and enhance germination of Gardner saltbush seeds.

Factor	Treatment Within Factor
Prechill (Stratification)	No Prechill
	2 week Prechill
	4 week Prechill
Washing	No Washing
	1 Hour Washing
	24 Hours Washing
Scarification	No Scarification
	20 Second Scarification

environmental parameters to further enhance germination. These exogenous substances are referred to as secondary germination enhancers (Young and Evans 1978). In this study several hormones and growth regulators were applied to the seeds (Table 2). The determination of secondary germination enhancers in our studies follows work by Pearson (1957), Khan et al. (1973), McDonough (1976), McDonald and Khan (1977), Roberts and Smith (1977) and Young et al. (1978).

1.B.2.d. Laboratory germination conditions. Germination was conducted in the laboratory by imbibing seeds on blotter paper in plastic germination trays. Trays were placed in a germinator alternating 24°C-light (16 hours)/13°C-dark (8 hours). Under ideal moisture conditions (distilled water) Beadle (1952), Eddleman (1978) and Osmond et al. (1980) found germination of Atriplex species declined significantly at temperatures from 25-35°C. Eddleman (1978) and McLean (1953) both recommended an alternating warm/cold temperature cycle for best Nuttall saltbush germination.

1.B.2.e. Parameters measured. Parameters measured in these experiments were cumulative germination (based on percent of total seed) and germination rate.

Treatments were compared using Duncan's Multiple Range Test to separate means at the 5 percent probability level.

1.B.3. Results and Discussion

1.B.3.a Environmental simulation treatment effects. Effects of dry storage time (dry storage afterripening), prechill, washing, and scarification on cumulative percent germination of "Knobs" (1980) seed

Table 2. Exogenous hormones and growth regulators applied[1/] to unscarified Gardner saltbush seeds.

Hormones:
Gibberellic Acid (GA_3) 30 M (10 ppm)
Growth Regulators:
KNO_2 0.2% (19.8mM)
Methylene Blue 30mM
Ferric Chloride 1.0%
Thiourea 3.0% (.394M)
2-Mercapto-ethanol 30 M
Dithiothreitol 30 M

[1/] Application was by water infusion.

are shown in Tables 3 and 4. Some points regarding these data follow:

(1) Dry Storage Time - The simple effect of time combined over all treatments in the factorial is shown in Table 4. No differences in germination were obtained between 2 and 5 months post-harvest, but a significant increase was obtained at 15 months post-harvest, indicating some degree of dry storage afterripening occurred.

(2) Prechill - In unscarified seed, 4 weeks prechill significantly enhanced germination at all post-harvest dates (treatment 1 vs. 7 in Table 3). Two weeks prechill was not effective in unscarified seed at any post-harvest date (treatment 1 vs. 4, Table 3).

The response to prechill was markedly different for scarified seed than unscarified seed. Four weeks prechill was not effective in 2 month post-harvest scarified seed, but increased in effect as the seed aged (treatment 10 vs. 16, Table 3). Apparently dry storage afterripened seeds are more sensitive to the combined effects of prechill with scarification than to prechill alone.

Two weeks prechill was not effective in enhancing germination except in 15 month post-harvest, scarified seed (treatments 1 vs. 4 and 10 vs. 13, Table 3). Dry storage afterripening seems to reduce the length of prechill requirement, but this is only apparent in scarified seed.

(3) Washing - The 24 hour wash period generally yielded greater germination than 1 hour wash (treatments 1 vs. 2 and 3, and treatments 10 vs. 11 and 12, Table 3). This difference became more significant in older seeds that were scarified.

Table 3. Effects of afterripening (months post-harvest) on germination response of Atriplex gardneri seeds to environmentally related seed treatments scarification, prechill, and washing.

Seed Treatments		PERCENT CUMULATIVE GERMINATION[1/] Seed Population Type; Year of Collection; Months Post-Harvest "Knobs" (1980) 2	5	15
No Scarification				
1. No Prechill	No Wash	6 g[2/]	4 f	16 g
2.	1 Hour Wash	8 fg	4 f	15 g
3.	24 Hour Wash	8 fg	11 de	20 fg
4. 2 Week Prechill	No Wash	9 efg	6 ef	17 g
5.	1 Hour Wash	8 fg	11 de	21 fg
6.	24 Hour Wash	10 efg	11 de	25 ef
7. 4 Week Prechill	No Wash	14 def	11 de	30 cde
8.	1 Hour Wash	13 def	9 ef	29 de
9.	24 Hour Wash	14 def	16 cd	33 bcd
Scarification				
10. No Prechill	No Wash	16 cde	17 cd	17 g
11.	1 Hour Wash	19 cd	17 cd	25 ef
12.	24 Hour Wash	15 cdef	28 a	35 bcd
13. 2 Week Prechill	No Wash	15 cdef	16 cd	32 cd
14.	1 Hour Wash	21 bc	19 bc	35 bcd
15.	24 Hour Wash	28 a	28 a	46 a
16. 4 Week Prechill	No Wash	21 bc	24 ab	36 bc
17.	1 Hour Wash	26 ab	28 a	39 b
18.	24 Hour Wash	30 a	28 a	50 a

[1/] Seeds germinated for 6 weeks in cycling 13°C-dark (8 hours)/- 24°C-light (16 hours).

[2/] Means within each column having similar letters are not significantly different at $P < 0.05$.

Table 4. Effect of dry storage time (afterripening) on germination of Knobs (1980) Gardner saltbush seed.

Months Post-Harvest	Cumulative Percent Germination (of total seed)		
	All Treatments[1/]	Unscarified Treatments	Scarified Treatments
2	16 b[2/]	10 b	22 b
5	16 b	9 b	23 b
15	29 a	23 a	35 a

[1/] Data were obtained by averaging all treatment means within a post-harvest period from Table (3); N = (2)(3)(3)(6) = 108. All unscarified treatment means and all scarified treatments means within a post-harvest date were averaged in column 2 and 3; N = (3)(3)(6) = 54.

[2/] Means within a column with similar letters are not significantly different at $P < 0.05$.

(4) Scarification - The simple effect of scarification, when combined over all prechill and wash treatments appears to show that scarification alone enhances germination (Table 4). However, in Table 3 it can be seen that scarification in the absence of wash and prechill only marginally enhances germination of 2 month post-harvest seed (6% to 16%) and 5 month post-harvest seed (4% to 17%), and does not affect 15 month post-harvest seed (16% to 17%).

The effect of 24 hours wash is dramatically increased in seeds that have been scarified (see treatments 3 vs. 12 in 5 and 15 month post-harvest seeds, Table 3). One hour wash is only effective in 15 month post-harvest seeds that have been scarified (treatments 2 vs. 11, Table 3). Prechill effects were enhanced by scarification but not to the degree that washing was. It appears that scarification acts to enhance the effects of other treatments such as wash and prechill rather than stimulate germination by itself.

(5) Physiological implications - Maximum viability of total seed in this study was 54 percent. Results show that Gardner saltbush seeds are highly dormant without treatment (6 percent germination, treatment 1, Table 3) and that dormancy can be completely broken (50 percent germination) using a combination of 15 months dry storage, 4 weeks prechill, 24 hours wash, and scarification (treatment 18, Table 3). It is important to note that both prechill and washing are necessary for complete removal of dormancy. Prechill without washing under scarification and 15 month dry storage conditions yields only 36 percent germination (Treatment 16, Table 3). Similarly, washing without prechill yields only 35 percent germination (Treatment 12, Table 3) .

Prechill and washing together, however, yield 50 percent germination (Treatment 18, Table 3). These results suggest that complete dormancy removal may not be possible unless physiological embryonic development from prechill occurs in conjunction with (seedcoat, bracteole and/or embryo) inhibitor removal from washing.

(6) Effects on germination rate - Cumulative percent germination was plotted as a function of time in the germinator. The number of days required to reach 90 percent of total germination (T_{90}) is shown for selected treatments in Table 5. Germination rates decreased in 2 month post-harvest seeds when they were washed 24 hours, but increased when these seeds were washed and scarified. The combination of 15 months dry storage, prechill, 24 hours washing, and scarification dramatically increased germination rates.

Our results agree with Eddleman (1978) who found that normal, untreated Nuttall saltbush germination was very slow, with most taking place over a 2 month period. Treating seed, however, affects germination rates such that it may be possible for a revegetation specialist to manipulate the germination rate for specific environmental conditions and thereby increase the likelihood for successful stand establishment.

1.B.3.b. Secondary germination enhancement effects. Hormones and growth regulators are suspected to act on embryo dormancy. Gibberellic Acid is a general substitute for prechill (Young and Evans 1981). Roberts and Smith (1977) suggest that to overcome dormancy, respiration in the embryo must shift from normal glycolysis to the pentose phosphate (PP) shunt pathway. Hydrogen acceptors (oxidizing agents) such as KNO_2

Table 5. Germination rates as affected by seed treatments.

Treatment		Germination Rate T_{90} [1/]		
		Month's Post-Harvest		
		No Scarification	Scarification	
		2	2	15
No Prechill	No Wash	28 (6) [2/]		
	24 Hr. Wash	37 (8)	20 (15)	17 (35)
2 Week P.C.	No Wash	25 (9)		
	24 Hr. Wash	39 (10)	18 (28)	8 (46)
4 Week P.C.	No Wash	21 (14)		
	24 Hr. Wash	26 (14)	14 (30)	5 (50)

[1/] T_{90} = the number of days required to achieve 90 percent of total cumulative germination.

[2/] Values shown in parentheses are percent cumulative germination (of total seed). These values are the same as shown in Table (3).

and methylene blue, and sulfhydryl compounds (dithiothreitol, mercapto-ethanol, thiourea) are suspected to act in such a way as to shift glycolysis to the PP shunt.

Secondary germination enhancement met with limited success in this study. Only water infusion of 2-mercapto-ethanol increased germination significantly over the control in both non-chilled and prechilled seeds (Table 5).

Seed in this study was uncertified and this may have restricted penetration of regulators into the embryo.

1.B.3.c. Characterization of Dormancy. It is well documented that bracteoles and/or seedcoats in Atriplex spp. contain salts and/or other compounds which inhibit germination either by creating a negative water potential against water uptake, or by directly inhibiting biochemical aspects of the germination process (Beadle 1932, Osmond et al. 1969). It is also well known that leaching Atriplex seeds with water enhances germination and this may be related to removal of seedcoat inhibitors. Our washing and scarification results imply that dormancy in Gardner saltbush may be seedcoat related.

Other results of this study strongly suggest that dormancy in this species may be embryo related as well. The effects of time in dry storage and prechill increased germination significantly. Both of these treatments have been associated with affecting embryo dormancy although the physiology is not well understood (Budrick and Cuzzort 1954, Lewis and Budrick 1977, Copeland 1976 (p 130), Khan 1977). The enhancement of washing effects by scarification and dry storage time suggests that

Table 6. Effects of hormones and growth regulators on germination of non-prechilled and prechilled Gardner saltbush seeds.

Treatment[2/]	Percent Cumulative Germination[1/]	
	No Prechill	Prechill
1. Control	6 def[3/]	8 bcde
2. KNO_2 (0.2%)	5 def	11 bcd
3. Methylene Blue 30mM	6 cdef	13 abc
4. $FeCl_3$ (1%)	6 def	6 cdef
5. Thiourea .394M (3%)	1 f	13 ab
6. Mercaptoethanol 30µM	13 abc	17 a
7. Dithiothreitol 30µM	4 def	7 cdef
8. Gibberellic $Acid_3$ 30µM	7 cdef	7 cdef

[1/] Seeds germinated in cycling 13°C (8 hours)/24°C (16 hours) for 6 weeks.

[2/] Seeds were imbibed in distilled water containing the chemical in the concentration denoted above.

[3/] Means followed by similar letters are not significantly different at $P < 0.05$.

germination inhibitors man be present in or near the embryo rather than in the bracteoles or seedcoat.

It should be noted that Young and Evans (1976) feel the mode of action for prechill may be seedcoat related in some cases such as in bitterbrush seed. The embryo may fail to germinate because of lack of oxygen diffusion through the seedcoat. In a cold, moist environment such as prechill conditions, more oxygen is soluble in water and the oxygen requirements of the embryo are lower.

Hormones and growth regulators generally act on embryo dormancy (Copeland 1976, Khan 1977). Our results were inconclusive in this area although we did detect some effects by mercapto-ethanol. Seedcoat factors probably inhibited uptake of regulators into the embryo in our study.

1.C. Big Sagebrush

1.C.1. Introduction

The literature shows that dormancy in big sagebrush (Artemisia tridentata Nutt.) is controlled by the pericarp and/or endosperm (McDonough and Harniss 1974). Light intensity also is an important factor (Weldon et al. 1959). Big sagebrush germinates well without prechill but germination increases and becomes more rapid with up to 60 days prechill at 3-5°C. However, germination decreases after 90 days prechill at 3-5°C (Vories 1981). Temperatures of 12° to 26°C yield the best big sagebrush germination (Sabo et al. 1979).

Germinative capacities of this species vary greatly from 0 to 94 percent germination in 15 to 100 days (Vories 1981).

1.C.2. Methods, Results, Discussion

In an experiment conducted under the same prechill and germinator conditions as in the Gardner saltbush study (Section I.B.2.c), it was found big sagebrush germinated well without prechill but did slightly better with 3 to 5 weeks of prechill (Table 7). Prechill also increased germination rates. Seeds used in this test were 95 percent viable (using 0.1 percent TZ).

It is recognized that establishment of big sagebrush on disturbed lands by seeding is a significant problem. In lieu of our germination results, it appears these problems may be more related to poor seedling vigor, perhaps involving the need for micro organismal symbiosis, rather than seed germination.

1.D. Rabbitbrush

1.D.1. Introduction

Rabbitbrush (Chrysothamnus spp.) germination has received much less research attention in the past than Atriplex spp. and Artemisia spp. Rabbitbrushes may have light and temperature factors involved in dormancy breakage. Light plays a major role in germination response of certain species and even subspecies, but has no effect in others (Sabo et al. 1979). Prechill treatments enhance germination with recommended temperatures from 1-4°C (Eddleman 1977). Temperatures above 28°C inhibit germination. Seedcoat dormancy is not a factor in rabbitbrush germination (Sabo et al. 1979).

Table 7. Effects of prechill on cumulative percent germination (of total seed) and germination rate in big sagebrush seeds.

Prechill Length	Cumulative Percent Germination	Days to T_{90}
Control	67	19[1/]
1 week	70	20
2 weeks	69	14
3 weeks	79	6
4 weeks	89	10
5 weeks	85	8
6 weeks	73	11
7 weeks	74	5

[1/] T_{90} = the number of days required to achieve 90 percent of total cumulative percent germination.

1.D.2. Results and Discussion

In our studies, effects of prechill and light on germination of two species, Rubber rabbitbrush (C. nauseosus (Pall.) Britton) and Greene rabbitbrush (C. greenei (A. Gray) Greene), were observed (Table 8).

Effective prechill length in illuminated Rubber rabbitbrush seed ranged from 1 to 3 weeks. This range of effective prechill length in Rubber rabbitbrush was reduced in the absence of light, as only the 2 week prechill period had any effect. The range of effective prechill length was also very short in nonilluminated Greene rabbitbrush seeds.

Germination for both rabbitbrushes was generally very low. Viability tests determined that both rabbitbrush species were less than 30 percent viable.

1.E. Siberian Peashrub

1.E.1 Introduction

Siberian peashrub (Caragana arborescens Lam.) is presumed to have a deep embryo dormancy which can be broken with prechill periods as short as 12 days at 5°C (USDA 1974).

Siberian peashrub is a hard seeded legume and the seedcoat may have a role in inhibiting germination. Cram (1969) found that certain pesticides such as captan, thiram, and mercuric chloride increased germination possibly by inhibiting seed-borne disease.

Peashrub seeds apparently do not have a light requirement (USDA 1974).

Table 8. Effects of prechill and light on cumulative percent germination of Rubber rabbitbrush (Chna) and Greene rabbitbrush (Chgr).

Prechill Duration	Cumulative Percent Germination[1/] (of total seed)		
	With Light	No Light	
	Chna	Chna	Chgr
None	2	7	0
1 week	11	2	5
2 weeks	9	14	7
3 weeks	24	9	3
4 weeks	7	8	3
5 weeks	7	4	0
6 weeks	-	2	2

[1/] Seed germinated for 5 weeks in 13°C (8 hour)/24°C (16 hour) cycle.

1.E.2. Results and Discussion

Seed used in our study was collected from the same tree in Laramie, August, 1980. Effects of prechill and scarification were tested in October, 1980. Prechill temperature was 2°C.

Table 9 shows that Siberian peashrub seeds responded completely to 1 week prechill. Scarification was detrimental to germination. We noticed that scarified seeds had much more fungal contamination than unscarified seeds. Perhaps, this treatment facilitated release of seedcoat borne pathogens or penetration of pathogens into the embryo.

In an additional experiment it was noticed some peashrub seedcoats were spotted and mottled (like a pinto bean) and others were not. Ferguson (1967) found that spotting was slightly detrimental to germination of bitterbrush seed. We tested germination of mottled vs. unmottled peashrub seed without any prechill treatment and found unmottled seed had a 24 percent germination while mottled seed germinated only 11 percent. This was a statistically significant difference.

Ferguson (1967) detected some necrosis in the cotyledons of spotted bitterbrush seed and speculated it was caused by insect damage. The cotyledons of Siberian peashrub were not analyzed in this study.

Table 9. Effect of prechill and scarification on germination of Siberian peashrub seed. The seed was 2 months post-harvest.

Prechill Duration	Cumulative Percent Germination[1/] (of total seed)	
	No Scarification	Scarification
0	6 d[2/]	4 d
1 week	98 a	25 b
4 weeks	100 a	17 c

[1/] Seed germinated for 4 weeks in 13°C (8 hours)/24°C (16 hours) cycle.

[2/] Means followed by similar letters are not significantly different at $P < 0.05$.

SECTION 2

FIELD EMERGENCE AND ESTABLISHMENT OF GARDNER SALTBUSH BY SEEDING

Contents

2.A. Introduction

Much recent literature has contributed to the understanding of germination in native arid land seeds; most notably by Young and Evans in Nevada and McDonough in Utah. Most of this research has been restricted to laboratory conditions, however. A more pertinent area of native seed germination research may be emergence and survival of seedlings under natural environmental conditions. Seedling emergence from soil involves much more than the processes involved in emergence of the radicle and cotyledons from the testa. As Osmond et al. (1980, p. 169) succinctly stated: "it is the establishment phase rather than germination which is more vulnerable under natural conditions and, if this is so, the detailed physiology and biochemistry of germination is of somewhat academic interest."

The objective of research discussed in this chapter was to observe effects of germination enhancement treatments, as developed in the laboratory, on emergence of Gardner saltbush seedlings under natural conditions.

2.B. Preliminary Study To Modify Laboratory Developed Seed Treatments For Field Application

2.B.1. Introduction and Methods

Young and Evans (1981) noted that once seeds of most species are prechilled, they must be planted immediately for the germination enhancement to be effective. They further mention that if the prechilled seeds are dried, not only is the advantage gained by prechill lost, but the seeds become inviable. Seeding moist, prechilled seeds,

is mechanically impractical. Young and Evans (1981) conclude that prechilled seeds can only be seeded in a seedbed that is conducive to continued germination.

We observed effects of various periods of drying at room temperature following 3 week prechill and 24 hour wash treatments on Gardner saltbush laboratory blotter germination. "Red Desert" (1981) seed was used for this study (see Figure 1), due to an inadequate supply of the "Knobs" seed used in the laboratory studies discussed in Section 1.

2.B.2. Results and Discussion

Results in Table 10 show that the enhancement effect of prechill is not lost with a 24 or 48 hour drying period following treatment. Washed seeds it seems can be dried almost indefinitely without damaging the enhancement effect. Germination in seed dried for 10 days after washing was significantly higher than unwashed seeds in both scarification treatments.

This preliminary study showed that germination enhancement obtained from prechill and wash treatments was retained by seed allowed to air dry. This implies that treatments would not have to be applied immediately prior to planting to retain their benefit.

2.C. Field Emergence Studies

2.C.1. Introduction and Methods

Gardner saltbush "Red Desert" (1981) seeds were subjected to scarification, 3 week prechill, and 24 hour washing treatments and spring planted (1982) at two topsoiled mine reclamation sites in

Table 10. Effects of various drying periods following wash and prechill treatments on germination of unscarified and scarified Gardner saltbush ("Red Desert - 1981") seeds.

	Wash Treatment	Prechill Treatment	Percent Germination (8 Months Post-Harvest) No Scarification	Scarification
1.	No Wash	No Prechill	7ef[1/],(6)[2/]f	8e,(6)e
2.	No Wash	3 Week Prechill (No Dry)	8 def	13 d
3.	No Wash	3 Week Prechill (24 Hour Dry)	14 abc	18 bc
4.	No Wash	3 Week Prechill (48 Hour Dry)	(10) cde	(14) cd
5.	24 Hour Wash (24 Hour Dry)	No Prechill	11 bcde	17 cd
6.	24 Hour Wash (48 Hour Dry)	No Prechill	(12) abcd	(18) bc
7.	24 Hour Wash (96 Hour Dry)	No Prechill	15 a	26 a
8.	24 Hour Wash (240 Hour Dry)	No Prechill	(10) cde	(22) ab
9.	24 Hour Wash (24 Hour Dry)	3 Week Prechill (No Dry)	15 ab	27 a
10.	24 Hour Wash (24 Hour Dry)	3 Week Prechill (24 Hour Dry)	15 ab	22 ab
11.	24 Hour Wash (48 Hour Dry)	3 Week Prechill (48 Hour Dry)	(14) abc	(23) ab
12.	24 Hour Wash (96 Hour Dry)	3 Week Prechill (No Dry)	14 abc	25 a
13.	24 Hour Wash (96 Hour Dry)	3 Week Prechill (24 Hour Dry)	15 ab	19 bc

[1/] Means within each column having similar letters are not significantly different at $P < .05$.

[2/] Data in parentheses were obtained in a second experiment run concurrently with the first, but in a different germinator. Light and temperature conditions of the two germinators were identical at: 13°C-dark (8 hours)/24°C-light (16 hours). Seeds were germinated for 6 weeks.

Wyoming, and at the University of Wyoming research plots in Laramie. The two reclamation sites were at Bridger Coal, north of Point of Rocks, and a Wyo-Ben bentonite mine east of Thermopolis. In addition to seed treatments, effects of 1 cm vs. 3 cm planting depth were observed at the field sites.

Each treatment replication consisted of 350 seeds planted in a single row 6.4 m (21 ft.) long. Prechill and wash treatments were conducted in the laboratory and dried at 20-25°C for 12-15 hours prior to seeding.

The Laramie site was irrigated periodically for 1 month after seeding to determine emergence under non-limiting moisture conditions. The reclamation sites received no supplemental irrigation after seeding.

2.C.2. Results and Discussion

2.C.2.a. Laramie irrigated site. Emergence at this site was far below germination obtained in the laboratory using the same treatments (Table 11). Response to individual treatments differed substantially between laboratory blotter germination and field emergence. Combined scarification, wash, and prechill gave the best results in the lab (treatment 8, Table 11). The best treatment for the Laramie field site was prechill with scarification (treatment 7, Table 11). The wash treatment had little enhancing effect in the field and actually seemed to inhibit prechill effects in scarified seed (treatment 8, Table 11).

2.C.2.b. Thermopolis and Bridger Coal dryland sites. Emergence at both dryland sites was much lower than the Laramie irrigated site (Table 11 vs. Tables 12 and 13). Emergence was greater at Thermopolis than at Bridger. Thermopolis received more precipitation than Bridger in the

Table 11. Effects of seed treatments on Gardner Saltbush blotter germination and field emergence at the irrigated Laramie, Wyo. site, 1982.

Treatment[2/]	Seedling Number [1/]		
	Laboratory Blotter Germination	Planting Depth	
		1 cm	3 cm
1. Control	25	1	2
2. W	38	2	2
3. P	50	15	11
4. W + P	51	14	9
5. S	29	3	2
6. S + W	58	6	4
7. S + P	64	28	11
8. S + W + P	78	10	9

1/ Each mean based on number of seedlings either germinated (blotter paper) or emerged (field planting) per 350 seeds treated. Seeds were germinated for 6 weeks on germination blotter. Emergence data were obtained 8 weeks after the seeds were planted.

2/ Treatments: Control = untreated seed, W = 24 hour wash, P = 3 week prechill (stratification), S = 20 second scarification.

3/ Means within each column having similar letters are not significantly different at $P < 0.05$.

Table 12. Effects of seed treatments on Gardner saltbush emergence at Wyo-Ben Bentonite reclamation site near Thermopolis, Wyoming, 1982.

Treatment[2/]	Seedling Number[1/]	
	Planting Depth	
	1 cm	3 cm
1. Control	2	1
2. W	3	2
3. P	5	5
4. W + P	5	4
5. S	5	5
6. S + W	4	4
7. S + P	8	8
8. S + W + P	7	5

[1/] Each mean based on number of seedlings emerged per 350 seeds planted in a single row 6.4 m long. Emergence data were obtained 16 weeks after seeds were planted.

[2/] Treatments: Control = untreated seed, W = 24 hours wash, P = 3 week prechill (stratification), S = 20 second scarification.

Table 13. Effects of seed treatments on Gardner saltbush emergence at Bridger Coal reclamation site near Point of Rocks, Wyoming, 1982.

Treatment[2/]	Seedling Number[1/]	
	Planting Depth	
	1 cm	3 cm
1. Control	0	1
2. W	T[3/]	1
3. P	T	2
4. W + P	T	2
5. S	1	T
6. S + W	T	2
7. S + P	T	2
8. S + W + P	1	4

[1/] Each mean based on number of seedlings emerged per 350 seeds planted in a single row 6.4 m long. Emergence data were obtained 16 weeks after the seeds were planted.

[2/] Treatments: Control = untreated seed, W = 24 hours wash, P = 3 week prechill (stratification), S = 20 second scarification.

[3/] T = Trace; when average seedlings per row is 0 but $\geq$ 0.5.

spring and summer 1982. In addition, topsoil at Thermopolis had a greater water holding capacity than Bridger topsoil.

Response to individual seed treatments at Thermopolis generally resembled results at the Laramie site, with the best treatment being prechill plus scarification (Table 12). Results at Bridger were too meager to make many conclusions. However, it appeared the wash treatment when combined with prechill was more beneficial there than at Laramie or Thermopolis (treatment 8, Table 13).

2.C.2.c. Effect of seeding depth. Significantly more seedlings emerged when planted at 1 cm than at 3 cm under irrigation (Figure 4). This difference was not apparent on the dryland sites. In fact, at the most arid site (Bridger Coal) more seedlings emerged from the 3 cm planting depth.

2.C.2.d. Summary. Although spring seeding is recommended for Gardner saltbush (Eddleman 1978, Vories 1981), fall seeding of shrubs is the common practice at revegetation sites, so that natural prechill can occur. However, seed loss from runoff, environmental exposure, loss of viability through time, false starting, and rodent predation are problems encountered with fall seeding. This study showed that spring seeding with artificially prechilled seed can be successful.

Recommended planting depth for Gardner saltbush is 1.3 cm (.5 inches) (Vories 1981). McLean (1953) found emergence of Nuttall saltbush declined rapidly when seeds were planted below 1.3 cm. The size of seed food reserves is the principle factor which determines the depth to which seed may be buried and still become established (Osmond et al. 1980). Gardner saltbush reserves are, of course, very limited

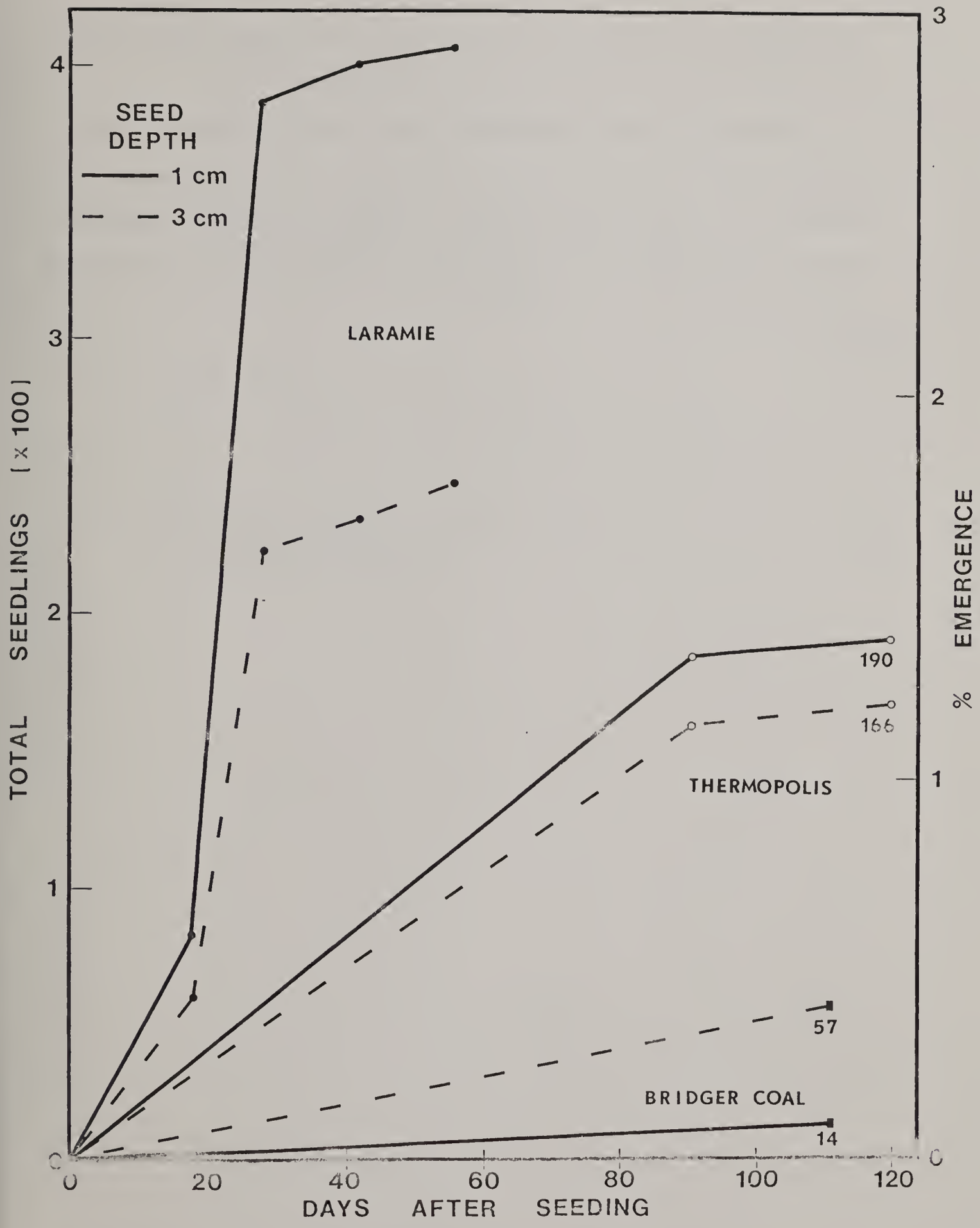

Figure 4. Effect of planting depth on Gardner saltbush emergence at three locations.

(see Figure 3). This study confirmed that 1 cm planting depth is best but deeper depths may have merit on extremely arid sites.

Tekrony and Hardin (1969) found that no single laboratory method gave an accurate measure of emergence behavior of sugarbeet seeds. This apparently is also true with Gardner saltbush as laboratory response to seed treatments did not, in many cases, correlate with field response to the same treatments.

SECTION 3

ECOLOGY OF GARDNER SALTBUSH SEEDS

Contents

3.A. Introduction

"Seed ecology" encompasses a broad spectrum of topics (Heydecker 1973). Our intent in this phase of the study was to observe aspects of Gardner saltbush seed ecology which may be of practical value to the revegetation specialist who is concerned with determining proper seed treatments and seeding rates. Subject areas discussed in this chapter are: (1) Effect of seed collection site on germination response to seed treatments, (2) Effect of year of collection of seeds from the same population on germination response to treatment, and (3) Fungal pathogenicity and its control.

3.B. Effect of Collection Site on Seed Germination Response

Three grades (3A, 3B, and 2B) of scarified and unscarified "Knobs" (1980) and "Rasmussen" (1980) seeds were washed 24 hours, prechilled 4 weeks, and then germinated in cycling 24°C-light (16 hours)/13°C-dark (8 hours) for 5 weeks. All seeds were 10 months post-harvest.

Results show that Knobs grade 3A and 3B seed had higher germination than Rasmussen seed under both scarification treatments, with differences being especially dramatic in unscarified seed (Table 14). Scarification removed differences in germination between the two populations of grade 2B seed.

Rasmussen seeds were collected from a low alkaline flat where Gardner saltbush grows in association with Greasewood (Sarcobatus vermiculatus) (see Figure 1 and Appendix B). Soils at this site are predominantly silty and alkaline. Knobs seeds, on the other hand, were from an upland site where soils are less alkaline (Appendix A). Perhaps

Table 14. Germination response of different populations and grades of Gardner saltbush seeds to scarification. All seeds were collected in August, 1980.

Seed Source	Grade	Percent Germination	
		No Scarification	Scarification
Knobs	3A	32 bc[1/]	46 a
Rasmussen	3A	6 e	39 b
Knobs	3B	36 bc	53 a
Rasmussen	3B	7 e	29 cd
Knobs	2B	22 d	33 bc
Rasmussen	2B	8 e	32 bc

1/Means followed by similar letters are not significantly different at $P < 0.05$.

the site on which the parent plants were growing influenced the chemical nature of the seeds and this affected germination capacity.

Additionally, in Table 14, Knobs grade 2B seeds had less overall germination than 3B seeds. This suggests that although grade 2B and 3B seeds weigh the same (as shown in Section 1.B.2.a.), most of the weight of 2B seeds may be in the bracteole rather than the embryo. Conversely, Rasmussen seeds had no differences in germination between grade 2B and 3B.

3.C. Effect of Year of Collection on Seed Germination Response

Response to treatment of seeds collected at the Knobs site in 1980 was similar to that of Knobs seed collected in 1981 except for a few significant differences (Table 15):

1) Overall germination was higher for all treatments in the 1981 seed, especially in treatment 9.
2) The 1981 seed was more responsive to the prechill treatments, being especially more responsive to 2 week prechill than the 1980 seed (treatment 1 vs. 4, Table 15).
3) Scarified 1981 seed showed a greater response to 1 hour wash than 1980 seed (treatment 1 vs. 2, Table 15).

3.D. Seed Fungal Pathogens and Their Control

3.D.1. Introduction and Methods

Virtually no research has been done in the area of fungal pathogen control in seedlings of plants endemic to Wyoming such as Gardner

Table 15. Effect of year of collection on germination response of Gardner Saltbush seeds to environmentally related seed treatments.

Seed Treatment		PERCENT CUMULATIVE GERMINATION 1/ Seed Population Type; Year of Collection; Months Post-Harvest			
		No Scarification		Scarification	
		"Knobs" (1980) 5	"Knobs" (1981) 5	"Knobs" (1980) 5	"Knobs" (1981) 5
1. No Prechill	No Wash	4 c 2/	8 e	17 c	13 d
2.	1 Hour Wash	4 c	10 de	17 c	25 c
3.	24 Hour Wash	11 ab	16 cd	28 a	31 c
4. 2 Week Prechill	No Wash	6 bc	15 cd	16 c	26 c
5.	1 Hour Wash	11 a	16 cd	19 bc	38 b
6.	24 Hour Wash	11 ab	26 ab	28 a	37 b
7. 4 Week Prechill	No Wash	11 ab	18 c	24 ab	28 c
8.	1 Hour Wash	9 bc	20 bc	28 a	40 b
9.	24 Hour Wash	16 a	27 a	28 a	53 a

1/ Seeds were germinated for 6 weeks in cycling 13°C (8 hours) - 24°C (16 hours).

2/ Means within each column having similar letters are not significantly different at $P < 0.05$.

saltbush. Seedling survival in the field may ultimately depend on whether the emerging radicle can survive fungal infection.

In our laboratory studies fungal contamination of emerging radicles was substantial. Even during prechill, contamination occurred on ungerminated seeds after about 2-3 weeks. The purpose of this study was to determine effects of two common fungicides, Thiram and Captan, on the germination and control of fungi associated with Gardner saltbush seeds. Ten month post-harvest "Knobs" (1980) 3B seed was used.

3.D.2. Results and Discussion

Thiram .005 g/g and .05 g/g controlled fungal infection in Gardner saltbush seed regardless of prechill or scarification treatment (Table 16). Captan .05 g/g had a similar effect. However, the lighter rate of Captan (.005 g/g) did not control infection in unscarified, prechilled seed, and was only marginally effective in the other scarification and prechill treatments.

Germination was not adversely affected by any fungicide treatment (Table 17). Thiram .005 g/g actually appeared to enhance germination in scarified seed (19 to 27 percent germination, Table 17). Thiram may act to replace prechill requirements in the seed, possibly in the same manner as sulfhydryl compounds like thiourea (see section 1.B.3.b.).

Table 16. Effects of Thiram and Captan fungicides on infection of scarified and stratified Atriplex gardneri seeds.

Treatment	Percent Fungal Infection[1/]			
	Unscarified Seed		Scarified Seed	
	No Prechill	Prechill[2/]	No Prechill	Prechill
No Fungicide	60 a[3/]	60 a	57 ab	64 a
Thiram .005 g/g seed	7 ef	15 ef	5 ef	11 ef
Thiram .05 g/g seed	3 f	3 f	2 f	7 ef
Captan .005 g/g seed	22 de	53 ab	33 cd	41 bc
Captan .05 g/g seed	7 ef	12 ef	9 ef	7 ef

[1/] A seed was considered infected if at least 25% of the seed coat or any portion of the emerging radicle was covered by mycelium, sporangia, conidia, or other fungal structures.

[2/] Stratification 4 weeks at 2°C. Seeds were germinated after stratification for 8 weeks in 10°-20°C (8 hour/16 hour cycle).

[3/] Means with similar letters are not significantly different ($P < 0.05$).

Table 17. Effects of Thiram and Captan fungicides on germination of scarified and stratified Atriplex garneri seeds.

Treatment	Percent Germination[1/]			
	Unscarified Seed		Scarified Seed	
	No Prechill	Prechill[2/]	No Prechill	Prechill
No Fungicide	6 f[3/]	18 de	19 de	31 ab
Thiram .005 g/g seed	9 f	23 cd	27 bc	35 a
Thiram .05 g/g seed	8 f	22 cde	23 cd	34 a
Captan .005 g/g seed	6 f	18 de	16 e	31 ab
Captan .05 g/g seed	6 f	19 de	21 cde	34 a

[1/] Seeds were germinated after stratification for 8 weeks in 10°-20°C (8 hour/16 hour cycle).

[2/] Stratification 4 weeks at 2°C.

[3/] Means with similar letters are not significantly different ($P < 0.05$).

CONCLUSIONS

Several broad conclusions can be made concerning results of this study:

(1) From an applied standpoint, simulated environmental treatments such as time in dry storage, prechill (stratification), washing, and scarification can be used in various combinations to obtain virtually any germination percent, including complete dormancy removal, and any germination rate in Gardner saltbush seeds under laboratory conditions.

(2) Physiologically, dormancy in Gardner saltbush seems to be both embryo and seedcoat related.

(3) Any germination study should compare laboratory obtained results with emergence and survival of similarly treated seeds under natural conditions. In this study laboratory results and field results were similar in many instances but markedly different in many others.

(4) Finally, seed source, year of collection, and fungal pathogenicity in native seeds are all important aspects of seed ecology which should not be overlooked during woody shrub revegetation. All too often establishment potential of a species is determined only by observation of laboratory obtained germination percentage values and this may not be entirely reliable.

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Appendix A

Figure 5. Specific location of the "Knobs" Gardner saltbush seed collection site (15 Km west of Rawlins, Wyoming). Collection site is represented by the black circle.

Appendix B

Figure 6. Specific locations of the "Rasmussen" (3 Km west of Wamsutter, Wyoming) and "Red Desert" (10 Km west of Wamsutter) Gardner saltbush seed collections sites. Collection sites are represented by black circles.

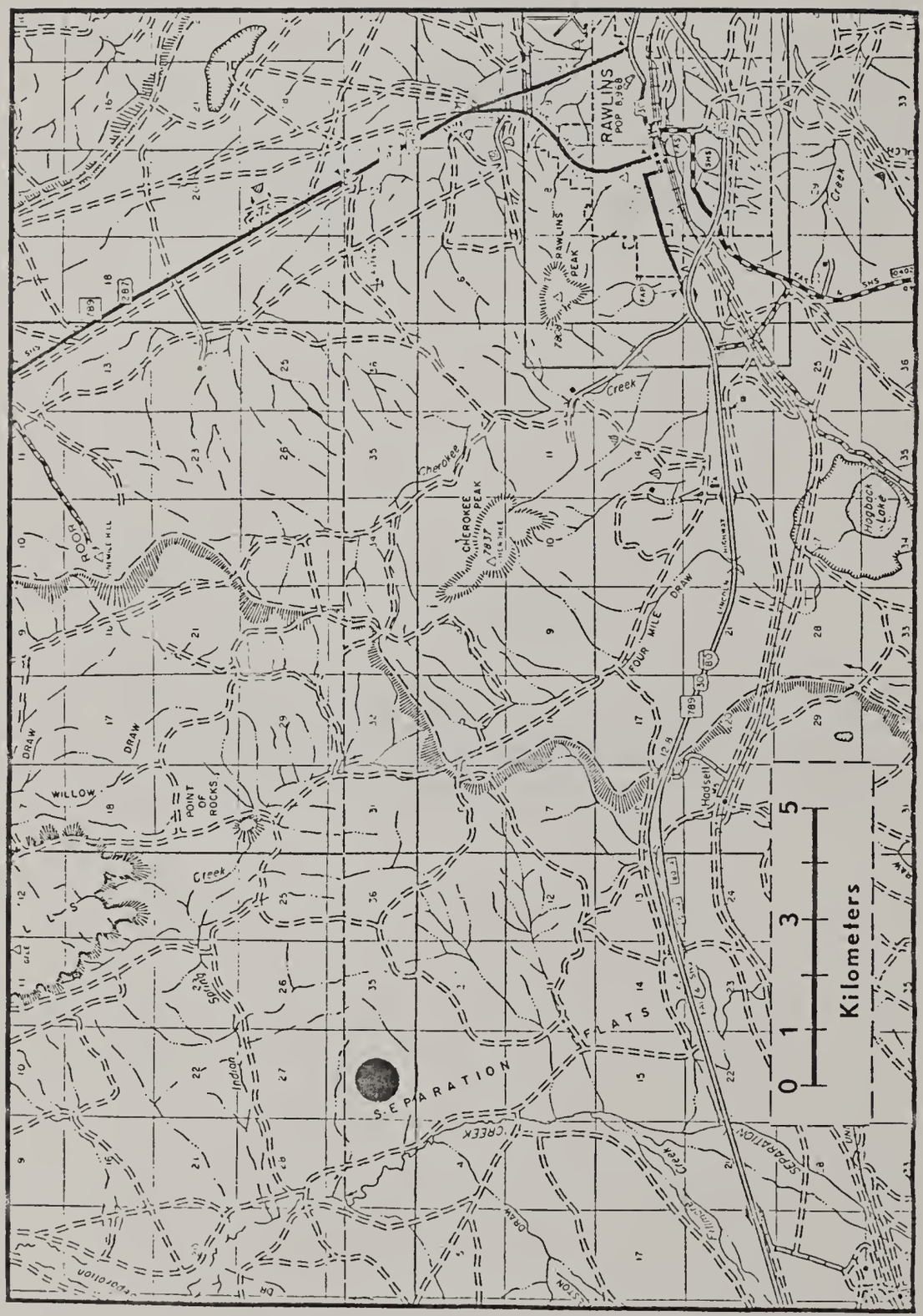

Figure 5. Specific location of the "Knobs" Gardner saltbush seed collection site (15 Km west of Rawlins, Wyoming). Collection site is represented by the black circle.

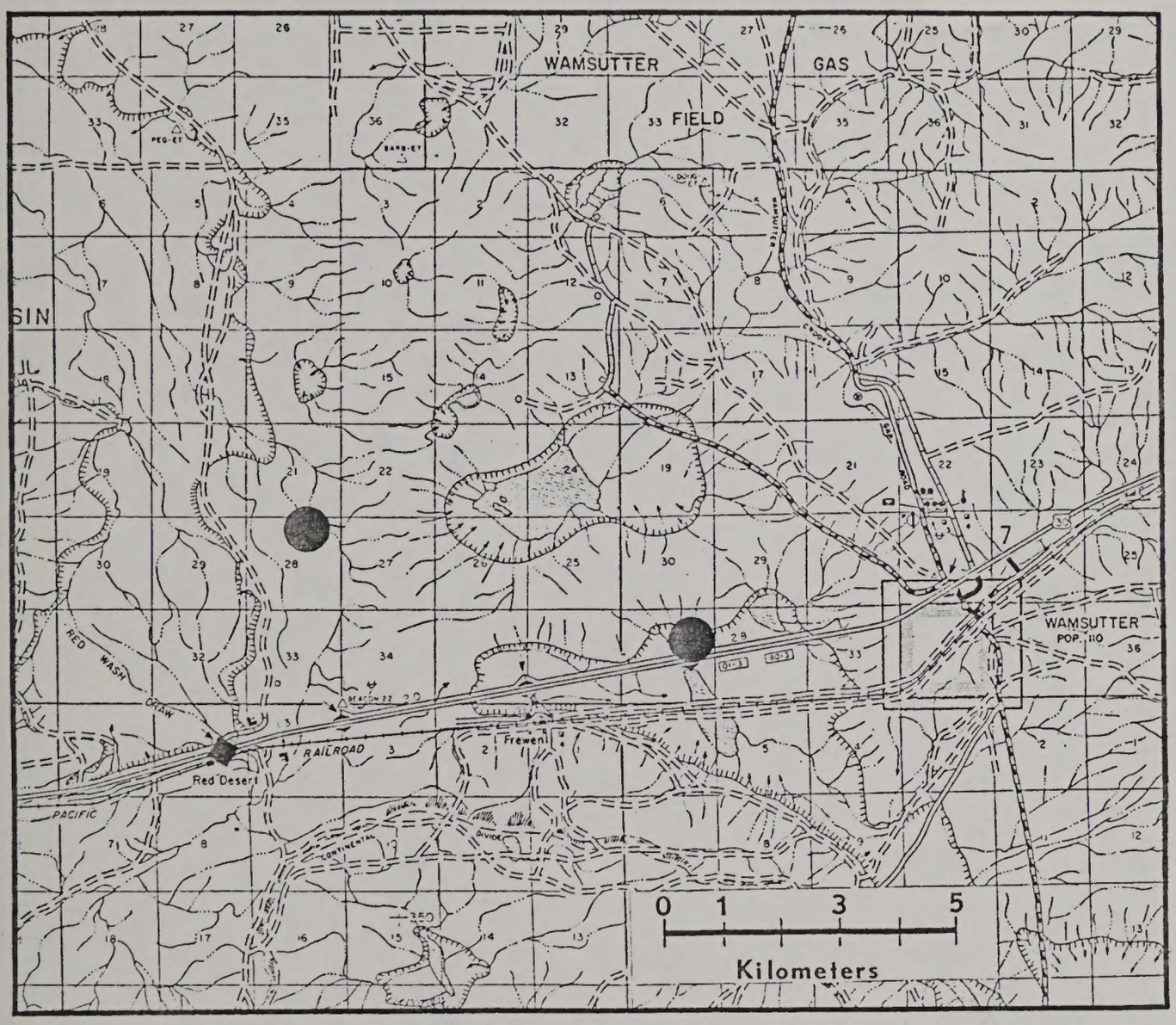

Figure 6. Specific locations of the "Rasmussen" (3 Km west of Wamsutter, Wyoming) and "Red Desert" (10 Km west of Wamsutter) Gardner saltbush seed collections sites. Collection sites are represented by black circles.